

Characterizing metribuzin herbicide tolerance in lentil

A Thesis Submitted to the College of

Graduate Studies and Research

In Partial Fulfillment of the Requirements

For the Degree of Master of Science

In the Department of Plant Sciences

Saskatoon

By
Kendra S. Meier

© Copyright Kendra Meier, December 2016. All rights reserved.

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work, or in their absence, by the Head of the Department or Dean of the College in which my thesis was done. It is understood that any copying of publication or use of the thesis, in whole or in part, for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Request for permission to copy or to make other use of material in this thesis in whole or in part should be addressed to:

Head of the Department of Plant Sciences
University of Saskatchewan
Saskatoon, Saskatchewan (S7N 5A8)

ABSTRACT

Worldwide, lentil (*Lens culinaris* Medik.) is an important pulse crop. Canada is the world-leading producer and exporter of lentils, with Saskatchewan alone exporting more than 2.0 Mt annually. Lentil is a poor competitor with both grasses and broad-leaved weeds, and chemical methods are the main form of control in mechanized broad acre production systems. Imidazolinone (Group 2) tolerance has been developed in lentil, but due to new resistance to this herbicide in weed populations, the use of metribuzin (MB) (Group 5, approved at 206 g a.i. ha⁻¹) has recently increased in western Canada. However, metribuzin can cause extensive damage to lentil crops under certain environmental conditions. The purpose of these studies was to develop strategies to improve tolerance to metribuzin in lentil through identifying potential genetic sources of improved tolerance and understanding the mode of inheritance of tolerance. Potential metribuzin tolerant F₂ populations were screened at 3x (618 g a.i ha⁻¹) rate of metribuzin application for improved resistance. Commercial lentil varieties were screened at 0x, 0.5x, 2x and 4x rates to evaluate tolerance to metribuzin to determine if natural variability occurs among genotypes and market classes. A mutagenized population of CDC Redberry was also screened at 10x (2060 g a.i ha⁻¹) and three putative tolerant selections were identified. Initial F₂ populations did not show improved metribuzin tolerance. There were significant differences of metribuzin tolerance between market classes, as well as between rates ($P < 0.0001$). Mutant and commercial genotype tolerance were quantified with dose response studies, indicating that PMBR-1 and 7529s had the largest ED₅₀. Lastly, a genetic study indicated that there was not the 3:1 susceptible to tolerant F₂ phenotypic ratio as hypothesized ($\chi^2(1, N=96) = 0.19, p < 0.05$), but rather a 15:1 F₂ phenotypic ratio of susceptible to tolerant.

ACKNOWLEDGEMENTS

I would like thank my supervisor Dr. Bert Vandenberg. His confidence and patience in me throughout this project has helped shaped my academic and professional career and leading to the completion of this thesis. Also, I am grateful for the guidance and friendship from my colleague Larn McMurray throughout this process. I would also like to offer thanks for the advice and input of my committee members, Drs. Chris Willenborg, Bunyamin Tar'an and Curtis Pozniak.

I am incredibly grateful to the entire Pulse team of the Crop Development Centre. I would not be able to complete my field and lab work without the support of Brent Barlow, Scott Ife, Jaret Horner and Stacey Wagenhoffer and Devini DeSilva. I would to acknowledge my family, Katherine Stanley, Jessica Weber, and Jennara Field as well as my fellow colleagues from Advanced Plant Breeding for their friendship and support. Lastly, I would not be able to complete this journey without the encouragement from Stephan.

Finally, I would like to express my appreciation for the Robert P. Knowles Scholarship for funding my education.

DEDICATION

I would like to dedicate this thesis to my dad, Garry Meier.
It is an honour to be referred as “Garry Meier’s Daughter”.

TABLE OF CONTENTS

PERMISSION TO USE.....	i
ABSTRACT	ii
ACKNOWLEDGEMENTS.....	iii
DEDICATION.....	iv
LIST OF TABLES.....	vii
LIST OF FIGURES	viii
LIST OF ABBREVIATIONS	xi
CHAPTER 1	1
1. INTRODUCTION	1
1.1.1 HYPOTHESES.....	2
1.1.2 EXPERIMENTAL OBJECTIVES	2
CHAPTER 2	
.....4	
2. LITERATURE REVIEW	4
2.1 INTRODUCTION OF LENTIL	4
2.2 TAXONOMY	4
2.3 GROWTH AND CULTIVATION OF LENTIL PRODUCTION IN CANADA	5
2.4 WEED COMPETITION EFFECTS ON LENTIL YIELD	6
2.5 HERBICIDE USE IN LENTIL	7
2.6 METRIBUZIN (MB).....	8
2.7 METRIBUZIN MODE OF ACTION AND LENTIL INJURY	9
2.8 METRIBUZIN TOLERANCE.....	11
2.9 PRELIMINARY GENETIC DATA FOR METRIBUZIN TOLERANCE IN LENTIL.....	12
CHAPTER 3	14
3. MATERIAL AND METHODS.....	14
3.1 EXPERIMENT 1: ASSESSING IMPROVED METRIBUZIN TOLERANCE IN F ₂ POPULATIONS	14
3.1.1 PLANT MATERIAL	14
3.1.2 EXPERIMENTAL DESIGN.....	15
3.1.3 METRIBUZIN APPLICATION.....	17
3.1.4 DATA ANALYSIS.....	17
3.1.5 STATISTICAL ANALYSIS	18

3.2 EXPERIMENT 2: ASSESSING METRIBUZIN TOLERANCE IN A MUTAGENIZED POPULATION OF CDC REDBERRY LENTIL.....	18
3.2.1 PLANT MATERIAL	18
3.2.2 METRIBUZIN HERBICIDE APPLICATION AND SCREENING	18
3.2.3 MUTANT CROSSING.....	19
3.2.4 DOSE RESPONSE STUDY OF MUTANT CDC REDBERRY LENTIL AND ITS PROGENY.....	19
3.2.5 MUTANT F ₂ POPULATION GENETIC STUDY	21
3.2.6 STATISTICAL ANALYSIS	21
3.2.6.1 DOSE RESPONSE STUDIES.....	21
3.2.6.2 F ₂ POPULATION GENETIC STUDY.....	21
3.3 EXPERIMENT 3 ASSESSING METRIBUZIN TOLERANCE IN COMMERCIAL LENTIL CULTIVARS.....	22
3.3.1 PLANT MATERIAL	22
3.3.2 INDOOR SCREENING.....	23
3.3.3 FIELD SCREENING.....	23
3.3.4 DATA COLLECTION	26
3.3.5 STATISTICAL ANALYSIS	26
CHAPTER 4.....	27
4. RESULTS AND DISCUSSION.....	27
4.1 EXPERIMENT 1: ASSESSING IMPROVED METRIBUZIN TOLERANCE IN F ₂ METRIBUZIN (MB) POPULATIONS.....	27
4.1.2 DISCUSSION.....	36
4.2 EXPERIMENT 2: ASSESSING METRIBUZIN TOLERANCE IN A MUTAGENIZED POPULATION OF CDC REDBERRY LENTIL.....	38
4.2.1 RESULTS OF DOSE RESPONSE STUDIES	38
4.2.2 F ₂ GENETIC STUDIES	43
4.2.3 DISCUSSION.....	43
4.3 EXPERIMENT 3: ASSESSING METRIBUZIN TOLERANCE IN COMMERCIAL LENTIL CULTIVARS.....	45
4.3.1 FALL 2014 OUTDOOR MB SCREENING.....	45
4.3.2 OUTLOOK VS. SPG SCREENING LOCATIONS.....	46
4.3.3 INDOOR VS. OUTDOOR STUDIES.....	49
4.3.4 DISCUSSION.....	59
CHAPTER 5	62
5. GENERAL DISCUSSION	62
LITERATURE CITED.....	64
APPENDIX 1 INDOOR METRIBUZIN SCREENING PROTOCOL.....	68
APPENDIX 2	73

LIST OF TABLES

Table 3.1 F2 lentil populations with parental lines available for 2013 field season	14
Table 3.2 Plant damage rating system based on the severity of plant damage from herbicide application	17
Table 3.3 Commercial genotypes used for metribuzin screening	22
Table 4.1 Effective doses metribuzin applied to mutant parental lines (CDC Redberry, PMBR-1, PMBR-2, PMBR-3) and F2 populations (7516as, 7525as, 7529s).....	38
Table 4.2 Effective doses of metribuzin when applied to three F2 populations of lentil....	40
Table 4.3 Effective doses of MB on F2 populations based on visual damage (percent damaged)	43
Table 4.4 Analysis of variance for the effect of MB rate (0.5x, 1x, 2x, 4x=824 g a.i. ha ⁻¹) on biomass reduction of market classes for lentil cultivars at Outlook and SPG.....	47
Table 4.5 Analysis of variance for the effect of MB rate on biomass reduction in field conditions of lentil market classes.....	57
Table 4.6 Analysis of variance for the effect of metribuzin rate on biomass in indoor conditions of lentil market classes.....	57

LIST OF FIGURES

Figure 2.1 Chemical structure of metribuzin	9
Figure 2.2 Metribuzin binds to protein B and outcompetes plastoquinone for the binding site and inhibits the electron transport chain (from Agarwal 2009).....	10
Figure 2.3 Range of metribuzin injury symptoms in lentil from Saskatchewan, spring 2013.	11
Figure 3.1 Example of the 2013 field experimental layout. The first three rows were unsprayed susceptible check VIR 421 and two market class checks (CDC Maxim and CDC Greenstar). Each set of three field passes (susceptible check, F2 populations, commercial check).....	16
Figure 3.2 The experimental layout of a replicate of the 2014 commercial lentil and lentil genotypes for field screening for MB tolerance. Columns represent varieties sown, and rows represent herbicide rates.	25
Figure 4.1 Survival (%) of MB tolerant parental lines (orange) and of elite breeding lines (blue) from the 2013 field season.....	28
Figure 4.2 Harvest index of MB tolerant parental lines (orange) and of elite breeding lines (blue) from the 2013 field season.	29
Figure 4.3 Mean Biomass (g) of MB tolerant parental lines (orange) and of elite breeding lines (blue) from the 2013 field season. Larger seeded varieties (3339-3, 3339-3b, 2275-17) and the MB-1 parental lines had the highest biomass.	30
Figure 4.4 Injury score of MB-1 populations and parents 1 week after MB herbicide application. VIR 421 was extremely damaged. MB-1 parental lines were least susceptible to MB.	31
Figure 4.5 Injury score of MB-2 F2 populations and parents 1 week after MB herbicide application. VIR 421 was the most susceptible towards MB.	32
Figure 4.6 Days after sowing to flowering for MB-1 F2 populations, MB tolerant parental lines and elite breeding lines. MB had little to no effect on initial flowering dates....	33
Figure 4.7 Days after sowing to flowering for MB-2 F2 F2 populations and parents, MB tolerant parental lines and elite breeding lines. MB had little to no effect on initial flowering dates.	34
Figure 4.8 Harvest index of MB-1 F2 populations, MB parental lines and elite breeding lines. MB parental lines and F2 populations had larger harvest indices than elite breeding lines and sprayed checks.	35

Figure 4.9 Harvest Index of MB-2 F2 populations MB parental lines and elite breeding lines. MB parental lines and F2 populations had larger harvest indices than elite breeding lines and sprayed checks.	36
Figure 4.10 Dose response curve for mutant parents and F2 populations (7529s, 7525as, 7516as) showing that increased rates of metribuzin decrease the amount of plant biomass. The curves indicate lentil biomass, and are all significant ($P<0.05$).....	39
Figure 4.11 Dose response curves of 3 F2 populations of lentil showing that increased rates of metribuzin decreased the amount of plant biomass. The curves indicate lentil biomass. All are significant ($P<0.05$). Lack of fit test is 0.9495.....	41
Figure 4.12 Dose response curve of 3 F2 populations of lentil showing that increased damage visual scores correspond with higher rates of metribuzin application. The curves indicate visual damage score (% necrosis), and are all significant ($P<0.05$). Lack of fit test is 0.9245.....	42
Figure 4.13 Mean percent biomass reduction of commercial cultivars and elite breeding lines sprayed with metribuzin at 4x (824 g a.i. ha ⁻¹) in Fall 2014.....	45
Figure 4.14 Mean percent biomass reduction by market classes for lentil cultivars sprayed with metribuzin at 4x (824 g a.i. ha ⁻¹) in Fall 2014.....	46
Figure 4.15 Sites used in in 2015 for screening metribuzin tolerance of lentil commercial cultivars and elite breeding lines. Both Outlook and SPG had irrigation set up. SPG had excessive straw residue.....	48
Figure 4.16 Mean percent biomass reduction of small red lentil commercial cultivars and elite breeding lines sprayed with MB in indoor conditions. 1x = 206 g a.i. ha ⁻¹	51
Figure 4.17 Mean percent biomass reduction of small green lentil commercial cultivars and elite breeding lines sprayed with MB in indoor conditions. 1x = 206 g a.i. ha ⁻¹	50
Figure 4.18 Mean percent biomass reduction of small red lentil commercial cultivars and elite breeding lines sprayed with MB in outdoor conditions. 1x = 206 g a.i. ha ⁻¹	52
Figure 4.19 Mean percent biomass reduction of large green lentil commercial lentil cultivars and elite breeding lines sprayed with MB in indoor conditions. 1x = 206 g a.i. ha ⁻¹	52
Figure 4.20 Mean percent biomass reduction of small green lentil commercial cultivars and elite breeding lines sprayed with MB in outdoor conditons. 1x = 206 g a.i. ha ⁻¹	53
Figure 4.21 Mean percent biomass reduction of large green lentil commercial cultivars and elite breeding lines sprayed with MB in outdoor conditions. 1x = 206 g a.i. ha ⁻¹	54

Figure 4.22 Photographs of typical injury observed in the indoor study of MB rate application involving different lentil market classes. Levels of plant injury are shown for CDC Maxim (small red), CDC Asterix (small green), CDC Greenstar (large green) and VIR 421	54
Figure 4.23 Mean percentage of biomass reduction of lentil market classes sprayed with four rates of MB under field conditions ($1x=206 \text{ g a.i. ha}^{-1}$)	55
Figure 4.24 Mean percentage of biomass reduction of lentil market classes sprayed with four rates of MB in indoor conditions ($1x=206 \text{ g a.i. ha}^{-1}$)	56
Figure 4.25 This photo shows two plants within the same pot of the same spray treatment and genotype, but displaying different symptoms in response to metribuzin application. This could be due to heterogeneity of metribuzin tolerance within genotypes.....	58
Figure A.2.1 These four plants of CDC Greenstar were planted on the same day. The two plants on the left were grown in a 60/40 Sunshine Mix # 3 and sand mixture. The two plants on the right were grown in Profile® Greens Grade™.....	75

LIST OF ABBREVIATIONS

CDC	Crop Development Centre, Saskatoon, Saskatchewan
HR	Herbicide Resistant
NLL	Narrowed Leafed Lupin
MB	Metribuzin
SPG	Saskatchewan Pulse Growers
UofS	University of Saskatchewan

CHAPTER 1

1. INTRODUCTION

Herbicide applications are commonly used for weed control in lentil. In Western Canada, producers are looking for a wider range of chemical tools to help control the Group 2 herbicide resistant weeds in lentil production systems. Use of metribuzin (MB), the first registered post-emergent herbicide for lentil, declined after the introduction of Group 2 herbicides, because of problems of inconsistent crop injury. Its use has been increasing in recent years, but concerns remain about crop injury and potential associated yield loss under certain environmental conditions.

MB is a Group 5 pre- and post-emergent herbicide, used to control both broadleaf and grass weeds in several agriculture crops, including lentil. It is safe to spray MB on lentil, however if the herbicide reaches the roots, there is potential for extensive damage to the plant, from minor chlorosis to complete desiccation (Saskatchewan Ministry of Agriculture 2010). MB can cause severe injury to lentil crops. For example, when applied at the 5-6 node stage on a hot day, MB can cause damage to lentil that is seeded less than 5 cm deep, resulting in stunting and defoliation (Saskatchewan Ministry of Agriculture 2008).

Imidazolinone herbicide resistance was developed in lentil by conventional breeding methods (Beckie 2012). Since 2007, farmers have relied heavily on the Group 2 herbicides for broad leaf weed management in lentil fields (Beckie and Reboud 2009), but weed populations are rapidly developing resistance to imidazolinone herbicides (Beckie 2012). A means of combating the Group 2 resistant weeds is to breed lentil for a new type of herbicide tolerance, such as tolerance to MB.

This project was designed to develop strategies to assist in the process of breeding lentil for increased tolerance to MB. This will eventually reduce crop injury and subsequent yield loss due to MB damage. Increased MB tolerance will also give producers more herbicide options to diversify herbicide rotations for lentil production ultimately a means of combating Group 2 resistant weeds.

1.1 RESEARCH HYPOTHESES AND OBJECTIVES

1.1.1 HYPOTHESES

Hypothesis 1: Crosses between MB tolerant lines, and both highly susceptible genotypes and elite breeding lines, will result increased tolerance to MB.

Hypothesis 2: Mutagenized CDC Redberry lentil will be more tolerant to MB than current commercial checks at 3x (618 g a.i. ha⁻¹) rates of MB.

Hypothesis 3: Commercially available lentil cultivars will have different levels of tolerance to MB due to natural variation in genotypes.

1.1.2 EXPERIMENTAL OBJECTIVES

Objective 1 (Hypothesis 1): An experiment was conducted to determine inheritance patterns of MB tolerance in crosses between tolerant and susceptible genotypes for specific lentil crosses.

Objective 2 (Hypothesis 2): A dose response experiment was conducted to confirm MB tolerance in potential MB tolerant lines.

Objective 3 (Hypothesis 2): Field screening of mutagenized CDC Redberry was conducted to identify potential metribuzin tolerance, followed by confirmation in the phytotron, then crossed with CDC Redberry to conduct F₂ genetic studies to investigate the inheritance of metribuzin tolerance.

Objective 4 (Hypothesis 3): An experiment was designed for field and indoor conditions to determine if natural variation occurs among existing commercial genotypes and elite breeding lines.

Objective 5: A protocol was developed to screen lentil for metribuzin tolerance under indoor conditions that can will be comparable to screening under field conditions.

CHAPTER 2

2. LITERATURE REVIEW

2.1 INTRODUCTION OF LENTIL

Lens culinaris Medikus is one of the oldest cultivated crops in agricultural history. The cultivated lentil has been linked back to 7000 BC, growing along the Fertile Crescent (Ladizinsky 1979; Global Crop Diversity Trust 2008), where farmers selected for non-dehiscent pods, non-dormant seeds, larger seed size, erect stems, and a variety of seed coat and cotyledon colours (Global Crop Diversity Trust 2008). The Fertile Crescent region still produces a significant proportion of world lentil production. World lentil production increased from 2.8-4.0 Mt between 2000 and 2010 (Erskine 2009). Lentil is an important agriculture product of Canada, as now that Canada is the leading exporting country in the world with Saskatchewan producing more than 2.3 Mt of lentil in 2015 (Saskatchewan Ministry of Agriculture 2015).

2.2 TAXONOMY

The cultivated lentil is member of the *Vicieae* tribe of the plant family Leguminosae, or Fabaceae, commonly known as the legume family (Scrippa 2010). The taxonomic relationships within the genus *Lens* continue to evolve. There are seven described species or subspecies of lentil: *L. culinaris*, *L. orientalis*, *L. lamottei*, *L. tomentosus*, *L. ervoides* L, *odemensis* and *L. nigricans* (Davies et al. 2007). Recently, four gene pools were identified using genotyping-by-sequencing. The new organization of gene pools locates *L. culinaris*, *L. orientalis*, and *L. tomentosus* in the primary pool, *L.*

lamottei and *L. odemensis* in the secondary pool, *L. ervoides* in the tertiary pool, and *L. nigricans* in the quaternary gene pool (Wong et al 2015).

Elena Barulina, a leading lentil researcher in the early 20th century, described and classified 58 lentil varieties in the 1920s. Cultivated lentil was categorized into two subspecies based on seed size: *macrosperma* (6-9mm seed diameter) or *microsperma* (2-6 mm seed diameter) (Barulina 1930, Tullu et al. 2011). The *L. culinaris* ssp. *microsperma* type geographically evolved in eastern regions of lentil cultivation, where ssp. *macrosperma* developed in the Mediterranean and European regions (Barulina, 1930; Tullu et al. 2011).

2.3 GROWTH AND CULTIVATION OF LENTIL PRODUCTION IN CANADA

Lentil is an annual, self-pollinating, herbaceous diploid ($2n=14$), with an indeterminate growth habit. It is a cool season legume that may require environmental stresses to initiate seed set (McVicar et al. 2010). Lentil has a fine branching stem, and will rarely grow higher than 45 cm (Saskatchewan Pulse Growers 2000). If the emerged growing point is damaged, new shoots will grow from the vestigial leaf nodes below the soil surface (CFIA 2010). The first true leaf develops from the third node, and new nodes are produced every 3-5 days, depending on growing conditions (McVicar et al. 2010). Leaves form at nodes on stems and branches may have up to 15 pairs of pinnate leaflets. In Canada, first flowering typically occurs when the lentil plant has produced 11-13 nodes (CFIA 2010). At that stage flowers begin to appear on indeterminate peduncles at nodes throughout the canopy, typically up to 1-4 flowers per peduncle. After the pod inflates, small, lens-shaped seeds form in the flattened pods. Lentils have various seed

coat colors, ranging from white, green, tan, gray, brown and black, and may also have a range of seed coat patterns (Vandenberg and Slinkard 1990).

In western Canada, lentil is most productive in Dark Brown soils (McVicar et al. 2010). The crop is best adapted to soils with a pH 6.0-8.0 and has low tolerance to flooded, water-logged soils or to saline soils (CFIA 2010). Even though lentil is considered a drought tolerant crop, if there is inadequate moisture in the early seedling stage, the crop may demonstrate dwarfing, resulting in decreased canopy size and lower yield (CFIA 2010).

Lentil is typically seeded once soil temperature is above 5⁰C, from late April to mid-May. Producers tend to seed as early as possible since lentil can tolerate late spring frost (Saskatchewan Ministry of Agriculture 2010). Lentil is typically seeded 3-8 cm deep at rates of 65-90 kg ha⁻¹ (large-seeded varieties) or 35-50 kg ha⁻¹ (small seed varieties) (Saskatchewan Ministry of Agriculture 2010). No nitrogen needs to be applied during seeding if inoculated with the nitrogen fixing bacteria *Rhizobium leguminosarum*. Phosphorous is applied at 20-40 kg ha⁻¹ if required to improve lentil growth (McNeil and Materne 2007).

2.4 WEED COMPETITION EFFECTS ON LENTIL YIELD

Weed competition is the greatest factor affecting yield potential of lentil on the prairies. Research has shown that weeds have contributed to 84% of yield loss in western Canada (Swanton et al. 1993). Lentil crops are inadequately competitive against both grass and broad-leaved weeds that may cause on average a 14% yield loss (Swanton et al. 1993), presumably caused by the lentil crop's poor seeding vigor, short stature, low vigor, slow early seeding development and thin canopy (Balser 1981, Boerboom and

Young 1995, Fedoruk and Shirliffe, 2011). To maximize lentil grain yield, weed competition needs to be minimized using management tools such as herbicides.

2.5 HERBICIDE USE IN LENTIL

Herbicide applications are the most common solution for weed control, especially for large scale farming systems in developed countries (Brand et al. 2007). Lentil producers do have some chemical options to control weeds, mainly for grasses (Group 1 herbicides). Broadleaf weeds can be controlled with pre-emergence burn off applications of glyphosate. In western Canada, fall applications of ethalfluralin and trifluralin are possibilities (McVicar et al. 2010). Limited options are available for broadleaf weed control in post-emergent growing conditions.

In 2006, ClearfieldTM lentils were introduced and provided farmers a new tool to control broadleaf weeds in lentil fields. ClearfieldTM lentil varieties tolerate Group 2 imidazolinone (IMI) herbicides (imazamox and imazethapyr), which are acetolactosynthase (ALS) inhibitors. These herbicides inhibit the ALS enzyme and halt the formation the amino acids valine, leucine and isoleucine (Whitcomb, 1999). These herbicides have high efficacy, low toxicity, crop selectivity and persist in the environment, allowing for prolonged weed control post-application (Devine and Shukla 2000, Dekker and Duke 1995, Saari et al. 1994, Whitcomb 1999).

Due to the high efficacy nature of Group 2 herbicides, this also led to rapid development of herbicide resistant (HR) weeds. Currently the number and frequency of weed species with Group 2 resistance is higher than that of any other herbicide group (Heap 2014). This is in part due to repeated applications of Group 2 products on lentil crops, which have limited post emergent options, and ultimately results in selection for

HR weeds (Beckie et al 2013). To help decrease the growing impact of HR weeds, another herbicide chemistry option with a different mode of action is required for the herbicide rotations of lentil production systems.

2.6 METRIBUZIN (MB)

MB is a Group 5 pre- and post-emergent herbicide used to control both broadleaf and grass weeds (Figure 2.1). The herbicide is applied to several agriculture crops including potato, tomato, rapeseed, corn, lentil, cereals, and alfalfa (Saskatchewan Ministry of Agriculture 2013). MB was first registered as a pesticide in Canada in 1971 (Koepki-Hill et al. 2011), and is distributed by Bayer Crop Science (Sencor™) and United Phosphorus Inc. (Tricor) (Saskatchewan Ministry of Agriculture 2013). MB was registered on lentil in 1980 (Communications with Eric Johnson, February 2016).

MB use on lentil is currently approved for single application rate of 205 g a.i. ha⁻¹, or two applications of 106-143 g a.i. ha⁻¹ (Bayer CropScience Inc. 2011). MB should only be applied to lentil crops seeded 5 cm deep or more in soils with 4% or higher organic matter (Saskatchewan Ministry of Agriculture 2014). MB was once a widely used product but its use has declined in recent years due to costs, application time and unpredictable efficacy due to environmental conditions (Saskatchewan Ministry of Agriculture, 2014), and the increased use of Group 2 herbicides.

MB (C₈H₁₄N₄OS or 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one) is in from the triazinone chemical family, and acts both as a systemic and contact herbicide. MB efficacy of weed control is influenced by soil pH, temperature and microbial populations (Bank and Robinson 1982). The compound is moderately water soluble, allowing for rapid uptake into the xylem (Saskatchewan Ministry of Agriculture

2014, Stephanova et al. 2012). In soils, MB adsorption is variable, and is nearly immobile in soils with high organic matter content and high pH, while it leaves in basic soils with low organic content (Saskatchewan Ministry of Agriculture 2014).

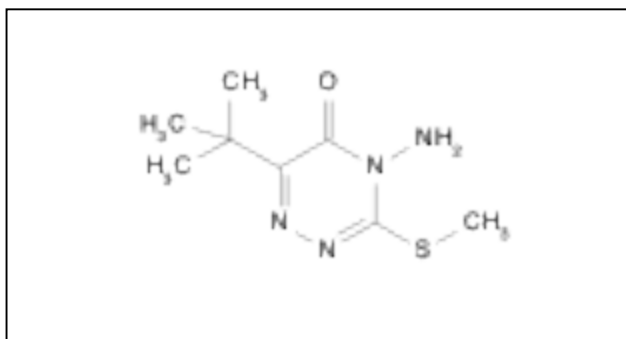


Figure 2.1. Chemical structure of metribuzin

2.7 METRIBUZIN MODE OF ACTION AND LENTIL INJURY

MB inhibits the electron transport chain during photosynthesis. MB will compete with plastoquinone at the plastoquinone binding site at the DI protein site of the photosystem II (PS II) complex, and disrupts the electron flow to photosystem I (PS I) (Stephanova et al. 2012). Without the electron transport chain, light energy cannot be harvested to produce hydrocarbons for the Calvin cycle in PS I (Figure 2.2). The excess buildup of electrons in PS II initiates damage to the plant membranes (Hall et al. 1999), causing production of toxic oxygen radicals due from the interactions between the excess electrons and molecular oxygen in the plant membranes (Hall et al. 1999). Even though the oxygen radicals are normally part of plant defense mechanisms, excess amounts result in the plant response that initiates the shutdown of PS II (Hall et al. 1999).

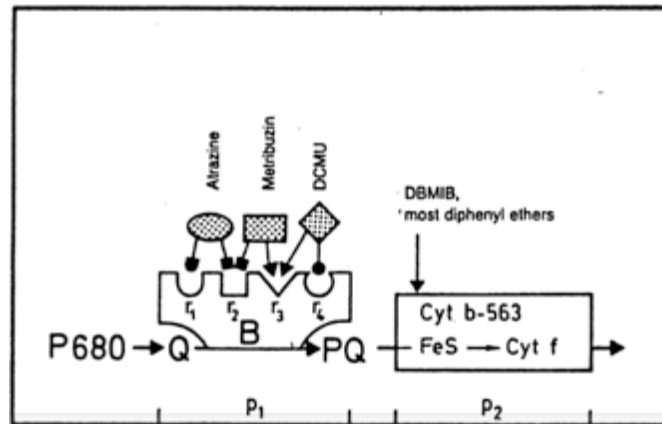


Figure 2.2 Metribuzin binds to protein B and outcompetes plastoquinone for the binding site and inhibits the electron transport chain (from Agarwal 2009)

When PSII has been shut down by MB, the plant will begin to show signs of desiccation and a reduction of chlorophyll and pigments (Hutchinson 2012). Due to high solubility in water, the PS II inhibitors travel mainly through the xylem. When applied to foliage, the herbicide is translocated to leaf margins, where rapid chlorosis and necrosis is first visible (Hall et al. 1999) (Figure 2.3). In grass weeds, the oldest leaves turn yellow and eventually the entire plant will desiccate, whereas broad leaf weeds show bleaching in the oldest leaves and death within days (Koepki-Hill et al. 2011). In comparison, systemic applications show the oldest leaves show first evidence of injury by wilting and yellowing, and then death occurs 7-10d later (Hall et al. 1999).



Figure 2.3. Range of metribuzin injury symptoms in lentil from Saskatchewan, spring 2013.

2.8 METRIBUZIN TOLERANCE

Narrow-leaved lupin (*Lupinus angustifolius* L.) is a grain legume commonly grown in Western Australia. Recent developments of improved MB tolerance in narrow-leaved lupin (NLL) cultivars showed that the inheritance of improved MB tolerance inheritance was derived from two induced mutants Tanjil-AZ-33 and Tanjil-AZ-55 (Si et al. 2009). These mutant lines were tolerant to applications of 800 g ha⁻¹ MB, and demonstrated no foliar damage (Si et al. 2009), whereas the wild type cv. Tanjil was completely defoliated. When the mutants were crossed with the susceptible wild type cv. Tanjil, a segregation ratio of 1:2:1 for highly tolerant:damaged:dead plants was observed. Progeny tests in F₂ and F₃ generations showed damaged plants were heterozygous and tolerant plants were homozygous, demonstrating the effect of a single semi-dominant gene conferred the MB tolerance (Si et al. 2009). When 4000 g ha⁻¹ of MB was applied

to segregating F₂ populations of NLL, 1/16 of the population had no herbicide damage, suggesting additive effects for MB tolerance inheritance.

Pan (et al. 2012) continued investigations of MB tolerance mechanisms in NLL. They sequenced the chloroplast *psbA* gene target site, and compared the two MB tolerant mutants (Tanjil-AZ-33 and Tanjil-AZ-55) with the susceptible wild type. By measuring photosynthetic activity, the MB tolerance mechanism in NLL was found to be a non-target mechanism involving P450 monooxygenase inhibitors (Pan et al. 2012).

2.9 PRELIMINARY GENETIC DATA FOR METRIBUZIN TOLERANCE IN LENTIL

Lentil genotypes with improved tolerance to MB were identified in the last few years in both Saskatchewan (Vandenberg, University of Saskatchewan, Saskatoon, Canada) and Australia (McMurray, SARDI, Adelaide, Australia). The UofS genotypes were designated as putative MB tolerant germplasm by using the designation MB. Crosses were made between the MB tolerant lines, and both highly susceptible genotypes and elite breeding lines. The F₂ populations were available for initial MB tolerance screening in summer 2013.

In 2012 field experiments, VIR 421 was observed to be more susceptible to MB lines compared to other lentil genotypes when the herbicide was applied at 3-4X (Vandenberg, unpublished data). This was confirmed in a subsequent indoor study where VIR 421 had the highest biomass reduction at all rates of MB application (0.25X, 0.5X, 1X, 2X and 4X) (Bo Langer UofS undergraduate thesis, 2013). Similar results of VIR 41 susceptibility to MB were also observed in experiments conducted as part of preliminary

research conducted to establish appropriate rates of MB application for indoor screening to represent field applications (Lulsdorf, unpublished).

CHAPTER 3

3. MATERIAL AND METHODS

3.1 EXPERIMENT 1: ASSESSING IMPROVED METRIBUZIN TOLERANCE IN F₂ POPULATIONS

3.1.1 PLANT MATERIAL

Two genotypes with apparent improved MB tolerance based on field screening were selected from preliminary trials conducted over the past few years. The study used F₂ seed produced from crosses made between the MB tolerant lines (MB lines), and normal lentil genotypes and elite breeding lines and also the highly susceptible line (VIR 421) identified earlier (Table 3.1). The parental lines and 13 MB F₂ crosses were compared with MB susceptible controls which included VIR 421 and two widely grown commercial check cultivars CDC Maxim (small red type) and CDC Greenstar (large green type). Twelve different MB F₂ populations were compared with parents and checks

Table 3.1 F₂ lentil populations with parental lines available for 2013 field season

Male	Population	Female
3494-6 (CDC Marble)	6614S-1	MB1-3
3494-6	6615S-4	MB2-3
3305-7 (CDC QG-3)	6622S-1	MB1-4
3305-7 (CDC QG-3)	6623S-1	MB2-1
MB1-3	6624S-3	2861-15a (CDC Asterix)
02M-12	6629S-1	MB1-3
MB1-3	6657S-3	3339-3 (CDC Greenstar)
MB2-2	6659S-1	3339-3
MB1-4	6665S-1	2275-15 (CDC Greenstar)
MB2-3	6666S-1	2275-15 (CDC KR-1)
3674-30	6675S-1	MB1-2
MB1-1	6681S-1	VIR 421
MB2-2	6685S-1	VIR 421

3.1.2 EXPERIMENTAL DESIGN

Experiment 1 was conducted in the field at the SPG research location. Plots were established in the field at the SPG farm on May 28, 2013. F₂ populations were subdivided into lots of 50 seeds which were sown into 1 m² microplots drilled into tilled wheat stubble, which had ethalfuralin incorporated in Fall 2012. The first three rows of microplots in the experimental area consisted of one entire pass of each of the unsprayed check genotypes (VIR 421, CDC Maxim and CDC Greenstar) (Figure 3.1). Next to the unsprayed checks, strips of microplots of the same checks were planted adjacent to each side (one row of susceptible and one row of commercial check) of the F₂ segregating populations and parent material. Each microplot consisted of 50 seeds. There were sets of three microplots per F₂ populations (3 x 50seeds) which were preceded and followed by a single 50-seed microplot of the parents each segregating F₂ population. The design was set up in sets of triplets (susceptible check, 3x50 seeds of F₂ populations, commercial check) with space for sprayer access between the strips of microplots. This allowed the sprayer to maneuver between the strips of microplots without driving over them, thereby minimizing the risks of uneven application.

Sprayer



Figure 3.1. Example of the 2013 field experimental layout. The first three rows were unsprayed susceptible check VIR 421 and two market class checks (CDC Maxim and CDC Greenstar). Each set of three field passes (susceptible check, F2 populations, commercial check)

3.1.3 METRIBUZIN APPLICATION

MB was applied at a 3x rate (618 g a.i. ha⁻¹) at the 5-6 node stage on June 19, 2013 at noon using a sprayer with air induction nozzle (110 015) at 9 gal/ac. The spray conditions were not ideal as it had rained had occurred for several days prior to application. The wind conditions were recorded at approximately 25 km/hr from the southwest. The 3x application rate was split into two separate applications of 1.5x applied separate passes in both west and east directions. Rain showers began again at 5 pm. There was a total of 6.9 mm of rainfall on June 19, 2013.

3.1.4 DATA ANALYSIS

The initial visual injury rating (based on whole plot) was recorded 10 d after herbicide application, followed by recovery ratings 21 d later after sowing. The injury score was based on the scale developed by Larn McMurray of University of Adelaide (Table 3.2). Each plant was then tagged and rated on a per plant basis for survival percentage, injury, days to flower, biomass and harvest index.

Table 3.2 Plant damage rating system based on the severity of plant damage from herbicide application

Score	Plant damage symptoms
0	No damage
1	Leaf chlorosis on less than 50% of leaves, no necrosis
2	Leaf chlorosis on 50 to 100% of leaves, no necrosis
3	All leaves showing chlorosis and less than 50% of leaves showing necrotic regions
4	All leaves showing chlorosis and regions of necrosis on 50 to 100% of leaves
5	All leaves necrotic and some plant death
6	All plants dead

Scale developed by Larn McMurray, SARDI, Adelaide, AUS

3.1.5 STATISTICAL ANALYSIS

Injury ratings, survival rates, flowering date, biomass and harvest index were recorded for each tagged plant in every microplot. To compare variation among parents and F₂ populations, the mean and standard errors were calculated from the data recorded for individual plants in each microplot, and presented in graphical form on a population basis.

3.2 EXPERIMENT 2: ASSESSING METRIBUZIN TOLERANCE IN A MUTAGENIZED POPULATION OF CDC REDBERRY LENTIL

3.2.1 PLANT MATERIAL

The study used M₃ generation seed of a CDC Redberry population produced from an application of sodium azide solution to the seed in collaboration with Dr. Victor Raboy of the USDA in Aberdeen, Idaho, USA. The initial mutagenized seed was planted and then bulked until the M₂ stage from which single plants were harvested. Seed of single plants were sent to New Zealand and sown as individual rows from which the M₃ seed was bulked. The M₃ progeny were sown in units of 120 seeds from each bulk source, and planted in microplots of approximately 1 m². A total of 1168 microplots were sown, for a total population of approximately 150,000 plants.

3.2.2 METRIBUZIN HERBICIDE APPLICATION AND SCREENING

MB was applied at the 5-6 node stage on June 19, 2014 at 4 pm in three separate passes using a 3x rate (618 g a.i. ha⁻¹) tank mix per application using a sprayer with air induction nozzle (110 015) at 9 gal/ac. The three initial passes (9x) were followed by a third 1x pass to achieve the final dosage application at 10x (2060 g a.i. ha⁻¹). A total of 18

surviving plants were tagged 10 days after spraying and caged for protection from the natural elements such as wind. The plants were dug out of the field 21 days after the 10x MB application and moved to the phytotron. Of these 18 plants, three were selected for further investigation based on increased MB tolerance, vigor and seed production. The mutant sources were designated PMBR-1, PMBR-2, PMBR-3. PMBR-2 and PMBR-3 are small red types with grey seed coats, similar to CDC Redberry. PMBR-1 is a large seeded lentil with a yellow cotyledon and green seed coat. It is unlikely that the different seed size and color is due to multiple mutations, but likely was a plant derived from a different seed source when a seed had fallen from the seeder.

3.2.3 MUTANT CROSSING

Seed of each of the three selected plants (PMBR-1, PMBR-2, PMBR-3) was multiplied to ensure enough seed was produced for crossing. In August 2014, crosses were made with CDC Redberry, SP1333 (a MB tolerant Australia source from Larn McMurray, SARDI), using the three potential MB tolerant lines as parents. Reciprocal crosses of F_1 plants were available by December 2014 and were used produce F_2 populations. The reciprocal F_1 plants were also crossed with both maternal and paternal sources. The F_2 populations of all crosses were available in March 2015 to begin genetic studies and dose response experiments.

3.2.4 DOSE RESPONSE STUDY OF MUTANT CDC REDBERRY LENTIL AND ITS PROGENY

A dose response study was used to quantify tolerance of the mutant parents and F_2 populations. The three mutant parents (PMBR-1, PMBR-2, PMBR-3) were screened in the same experiments with four check genotypes: SP1333, CDC Redberry, a potential

tolerant genotype ML-15 (Monika Lulsdorf, UofS) and VIR 421 (a known susceptible). The three F₂ populations tested were derived from crosses with CDC Redberry, where the mutant parent was the maternal plant, designated crosses as 7529s (PMBR-1 parent) 7516bS (PMBR-2 parent), and 7525s (PMBR-3 parent).

Plants were established in pairs in 10 cm² plastic pots. The plants were sprayed at the 5 node stage. Plant height was measured prior to MB application, then again at 7, 14 and 21 d after spraying. The plants were harvested 21 d after spray application, dried and weighed for biomass. The growth chamber was set at 14 h day length. Day temperature was set at 25 °C and night temperature was set at 15 °C. Percent injury was also recorded in 10% increments. 0% indicates no injury, where 100% indicates complete death.

Dose response experiments to quantify mutant parent tolerance were conducted in March and April 2015. There were 12 herbicide treatments plus a 0x control application with three replications. The rates used in the trials were 0x, 100g a.i. ha⁻¹, 150g a.i. ha⁻¹, 225g a.i. ha⁻¹, 337g a.i. ha⁻¹, 506g a.i. ha⁻¹, 759g a.i. ha⁻¹, 1138g a.i. ha⁻¹ and 1707g a.i. ha⁻¹, 2560g a.i. ha⁻¹, 3840g a.i. ha⁻¹, 5761g a.i. ha⁻¹, and 8641g a.i. ha⁻¹. These rates were the same as those used for MB dose response studies by Larn McMurray on Australian mutant lentil lines.

An initial dose response was conducted for the F₂ mutant hybrid populations in April 2015, concurrently with the mutant parent dose response study. From this initial screen, three crosses were selected for a subsequent dose response study in August 2015. The three F₂ populations (7529s (PMBR-1 parent), 7516bS (PMBR-2 parent), 7525s (PMBR-3 parent)) were screened in the same experiment with the mutant parents (PMBR-1, PMBR-2 and PMBR-3) and CDC Redberry.

3.2.5 MUTANT F₂ POPULATION GENETIC STUDY

The F₂ populations, 7516bs and 7529s were selected for an F₂ genetic study conducted in November 2015. The two mutant parents (PMBR-1, PMBR-2), CDC Redberry, CDC Greenstar and VIR 421 (known susceptible) were included in the study as repeated checks. This experiment took place in November 2015. For the F₂ populations, 100 seeds were planted plus 16 seeds were planted for each check. The entire experiment was sprayed with MB at 800 g a.i. ha⁻¹, the rate corresponding to ED₅₀ of the mutant populations from the August 2015 dose response study. Data were collected on the basis of plants being alive or dead after treatment.

3.2.6 STATISTICAL ANALYSIS

3.2.6.1 DOSE RESPONSE STUDIES

Statistical analysis for all dose response studies was conducted using the DRC package (Ritz and Streibig, 2005) from R (R Core 2015). The 4 parameter model had the best fit, and was used for all dose response analysis.

3.2.6.2 F₂ POPULATION GENETIC STUDY

A Chi-square (χ^2) test was conducted to test the potential fit of phenotypic segregation ratios for F₂ populations as a potential means of estimating gene frequency.

3.3 EXPERIMENT 3: ASSESSING METRIBUZIN TOLERANCE IN COMMERCIAL LENTIL CULTIVARS AND GENOTYPES

3.3.1 PLANT MATERIAL

Seed of 22 different genotypes was obtained for current, past and soon to be released commercial cultivars, known susceptible genotypes (VIR 421 and PBA Flash), and germplasm with increased tolerance to MB (SP1333 and MB1-4) (Table 3.3).

Table 3.3 Commercial genotypes used for metribuzin screening

Seed Source	Market Class	Origin
CDC Marble	French Green	Canada
CDC Greenland	Large Green	Canada
CDC Greenstar	Large Green	Canada
IBC 768	Large Green	Canada
SP1333	Large Green	Argentina (Australia)
3592-13	Small Green	Canada
CDC Asterix	Small Green	Canada
Eston	Small Green	Canada
CDC Viceroy	Small Green	Canada
CDC Invincible	Small Green	Canada
MB1-4	Small Green	Canada
VIR 421	Small Green	Russia
CDC Roxy	Small Red	Canada
CDC Cherie	Small Red	Canada
CDC Dazil	Small Red	Canada
CDC Impulse	Small Red	Canada
IBC 550 (CDC Proclaim)	Small Red	Canada
CDC KR-1	Large Red	Canada
CDC Maxim	Small Red	Canada
CDC Redberry	Small Red	Canada
CDC Scarlet	Small Red	Canada
PBA Flash	Small Red	Australia

3.3.2 INDOOR SCREENING

A total of 22 cultivars and genotypes were screened in a growth chamber (Convion, Winnipeg, MB) in the UofS in a RCBD design with three replications. The chamber was set at 14/10 h of day/night with the room temperature was set at 20 °C/15 °C. The light intensity was approximately 540 $\mu\text{mol m}^2 \text{s}^{-1}$ and humidity was maintained near 40%. Seeds of each genotype were scarified and sown into 4-inch plastic pots at 4 seeds per pot with a mixture of 60% Sunshine Mix 3 (Sun Gro Horticulture Canada Ltd. Vancouver, BC) and 40% commercial sand (Early's, Saskatoon, SK) which was homogenized using the soil mixer. Prior to sowing, each pot received 100ml of water. After sowing, pots were water every 3 d with 100ml of water containing quarter strength Hoagland's nutrient solution. The day before MB application, the plant population was thinned down to two plants per pot at similar node stages. The plants were sprayed in the laboratory with a with a single Even-Spray (Lechler, St. Charles, IL, USA) nozzle 8001 EVS delivering 100 L ha⁻¹ at 240 kPa. The plants were sprayed with MB at the 5-6 node stage at 5 rates (0, 103, 206, 412 and 824 g a.i. ha⁻¹). The next day, 50ml of water was applied to the surface of each pot to flush the chemical to the root system. The plants were harvested 21 d after spray application. Biomass samples were placed in envelopes and in the phytotron dryer for 10 days at 40°C.

3.3.3 FIELD SCREENING

Field screening was conducted at one site in fall 2014, and at two sites in summer 2015. The fall 2014 plots were located near the CDC field laboratory (52°07'27.43''N, 106°37'31.47''W) and were sown on September 15, 2014 into Dark Brown soil zone (organic matter 3.5-4.5%). In summer 2015, the experiment was repeated at the

Saskatchewan Pulse Growers (SPG) research land near Saskatoon, SK (52°03'28.7"N, 100°26'12.0"W) in the Dark Brown soil zone, and a location at a location east of Outlook SK (51°28'17.9"N, 107°00'16.3"W). The Outlook region (RM of Rudy No. 284) is at the southern edge of the Dark Brown soil zone, and at this location the experiment was deliberately sown in a sandy area to minimize MB interaction with organic matter and to maximize the damage from MB.

Long plots were sown into tilled soil with a row seeder. Three randomized replicates of 22 genotypes (Table 3.3) were sown in 12 m long plots at the rate of 100 seeds/plot. Within replicates, 5 rates of MB (0, 0.5x=103 g a.i. ha⁻¹, 1x=206 g a.i. ha⁻¹, 2x=412 g a.i. ha⁻¹ and 4x=824 g a.i. ha⁻¹) were sprayed horizontally across the plot (Figure 3.2). The spray application took place at the 5-6 node stage using a hand held sprayer at 100 L/ha using four 0.5 m spaced Air Mix 110-015 low-pressure nozzles at 240 kPa.

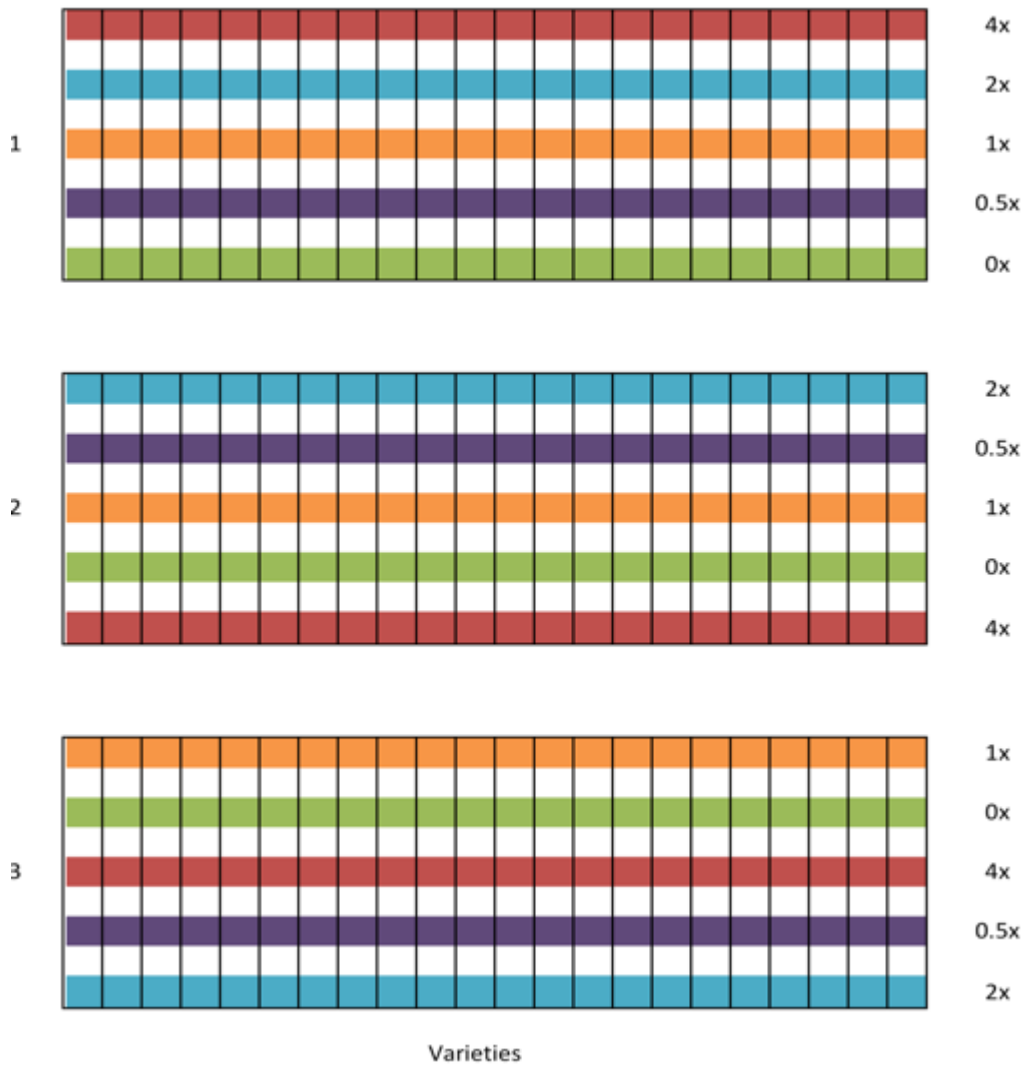


Figure 3.2. The experimental layout of a replicate of the 2014 commercial lentil and lentil genotypes for field screening for MB tolerance. Columns represent varieties sown, and rows represent herbicide rates. Varieties and herbicide rates are randomized in each rep.

3.3.4 DATA COLLECTION

In both outdoor and indoor settings, the plants were harvested 21 d after spray application then weighed for biomass. Biomass samples were placed in envelopes and in then phytotron dryer for 10 d at 40⁰C prior to weighing.

3.3.5 STATISTICAL ANALYSIS

In both indoor and outdoor experiments, percent necrosis and biomass were recorded for each genotype. To compare variation among genotypes mean and standard errors of the three reps were calculated and presented in graphical form. For both experiments, the means were compared using the PROC MIXED procedure at $P<0.05$ using SAS (SAS Institute Inc., 2014, Carey, NC, USA).

CHAPTER 4

4. RESULTS AND DISCUSSION

4.1 EXPERIMENT 1: ASSESSING IMPROVED METRIBUZIN TOLERANCE IN F₂ METRIBUZIN (MB) POPULATIONS

It was evident that extensive MB damage occurred in the 2013 field season, likely due to a combination of the 3x rate and environmental conditions that were conducive to MB injury on lentil. Evidence for a range of MB tolerance among genotypes was demonstrated by the variability of injury observed between parent lines and the variation in the response their segregating progeny. However, no statistics will be presented as these populations did not survive the MB application in 2014 and are no longer used in the lentil breeding program.

When comparing the MB tolerant lines with the elite breeding lines, it was evident that the MB lines had increased tolerance at the 3x MB application in terms of % survival (Figure 4.1). The cultivar CDC KR-1 (breeding line 2275-15) also appeared to have a higher than average survival after 3x MB application.

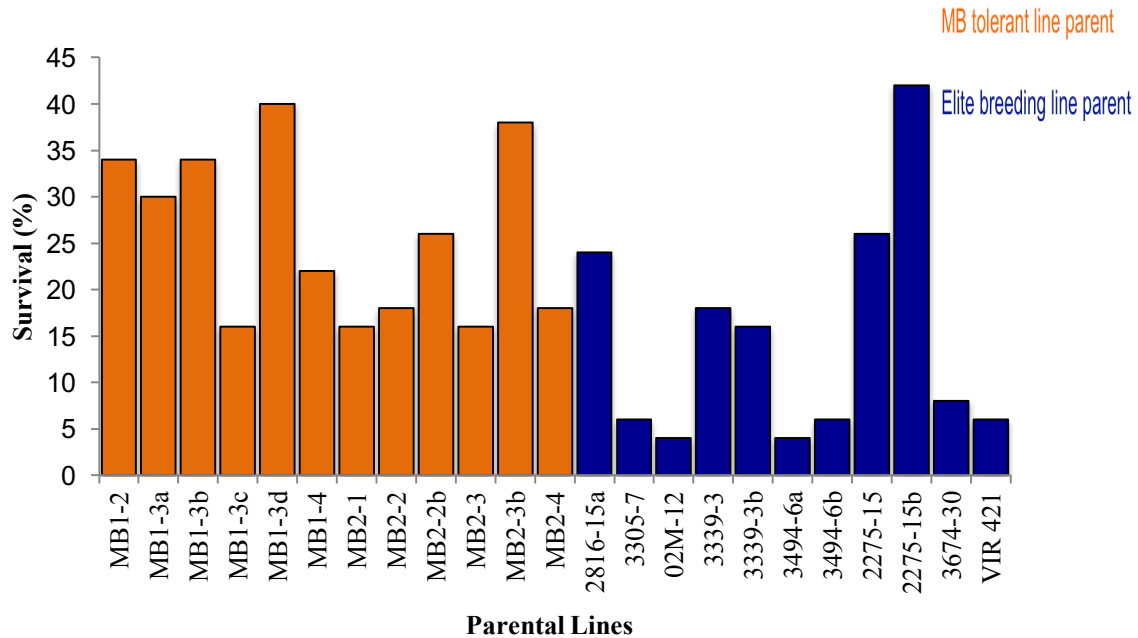


Figure 4.1 Survival (%) of MB tolerant parental lines (orange) and of elite breeding lines (blue) from the 2013 field season.

The harvest index data (Figure 4.2) indicated there were differences among the MB lines and among the elite breeding lines, and also between the two groups. This is likely due variation in genetic tolerance. Among the elite breeding lines, 2275-15 had the best tolerance to 3x MB. As a group, the MB lines demonstrated on average 11% higher survival compared to the elite breeding lines (Figure 4.2).

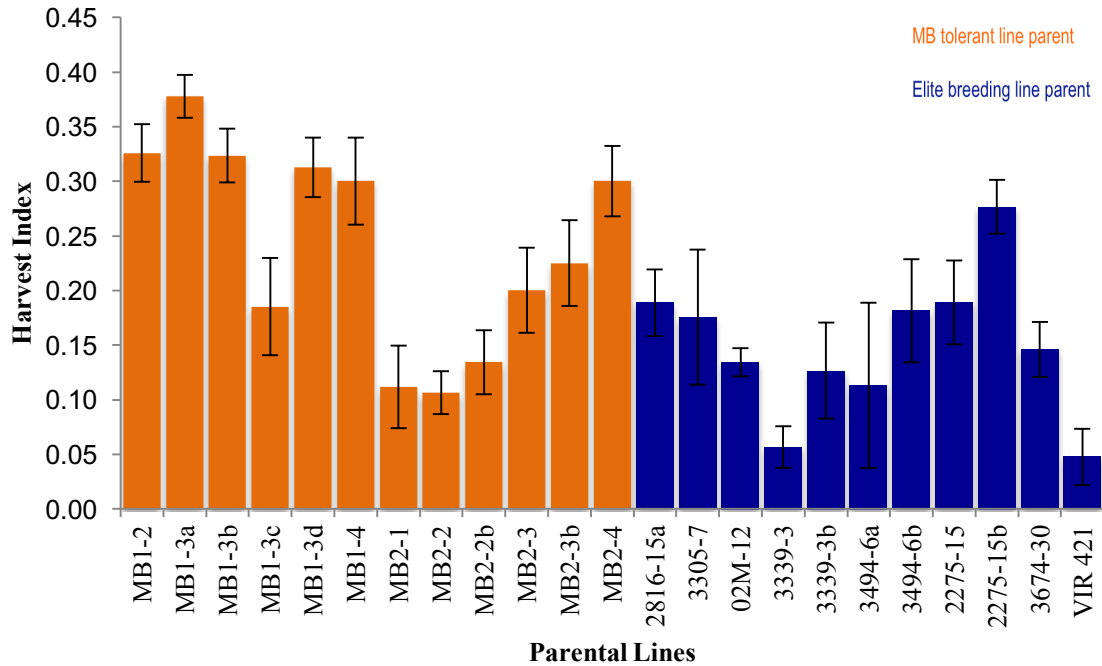


Figure 4.2 Harvest index of MB tolerant parental lines (orange) and of elite breeding lines (blue) from the 2013 field season.

This was not reflected in the biomass data (Figure 4.3), which showed that 3339-3 (CDC Greenstar), 3339-3b (CDC Greenstar derivative) and 2275-15 (CDC KR-1) had higher biomass than MB1-4, as they are medium-large seeded lentils. On average per plant, MB tolerant lines had a biomass of 16.9g, compared to the susceptible lines, which had on average 17.4g of biomass.

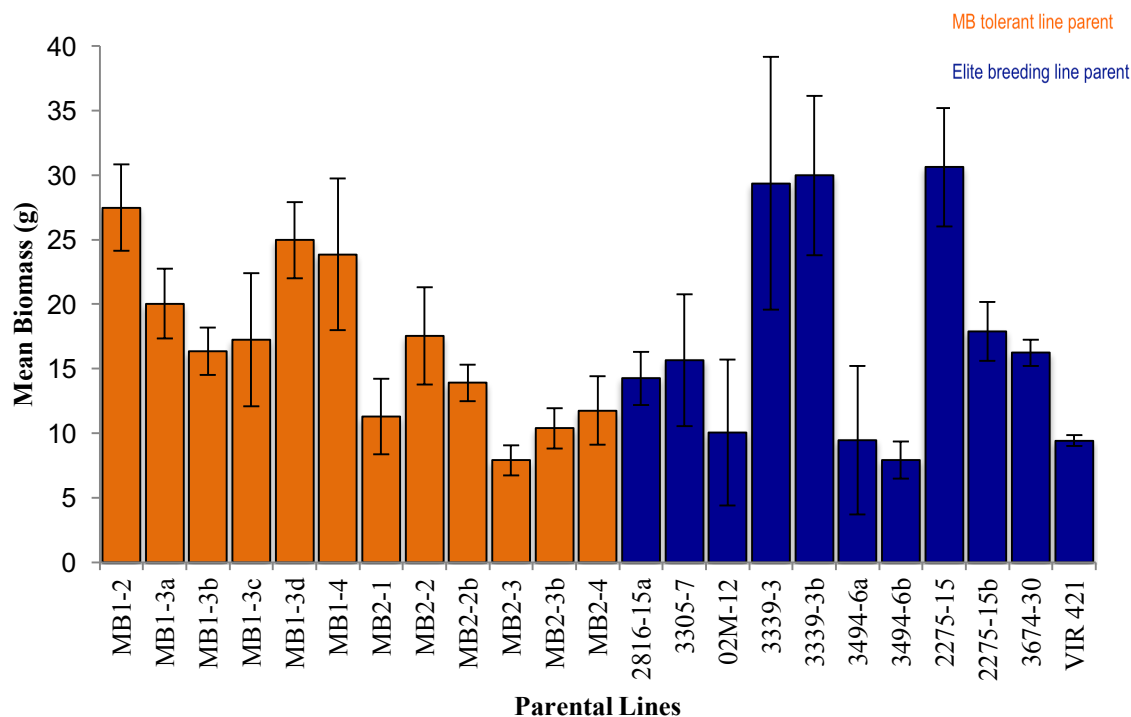


Figure 4.3 Mean biomass of plots (g) of MB tolerant parental lines (orange) and of elite breeding lines (blue) from the 2013 field season. Larger seeded varieties (3339-3, 3339-3b, 2275-17) and the MB-1 parental lines had the highest biomass.

The injury ratings were variable among parents and F_2 populations, again likely due to variation in genetic tolerance. This variation in genetic tolerance can be attributed to the environment, and that there is a genotype by environment interaction. The crosses with MB-1 parents had lower injury scores compared to the crosses made with MB-2 parents. The variation could have been due to environmental interactions (Figures 4.4 and 4.5). Also in the spray checks, CDC Greenstar and CDC Maxim had lower injury scores compared to VIR 421.

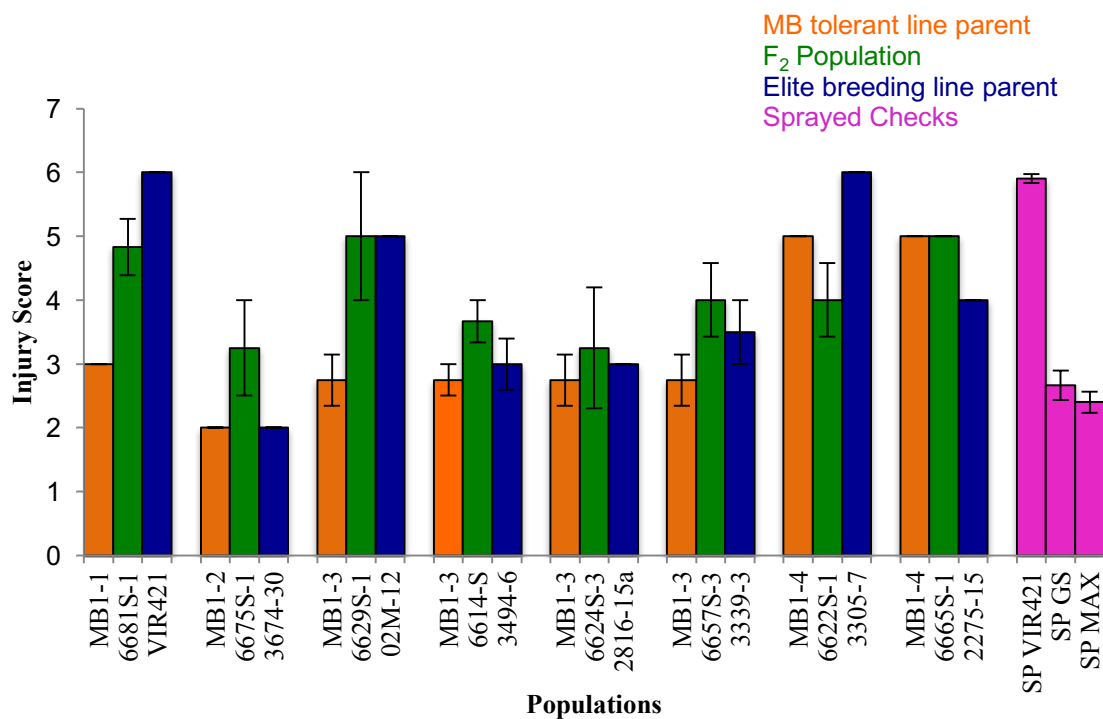


Figure 4.4 Injury score of MB-1 populations and parents 1 week after MB herbicide application. VIR 421 was extremely damaged. MB-1 parental lines were least susceptible to MB. 0 – no injury, 6 – complete necrosis. Scale derived from Larn McMurray (SARDI).

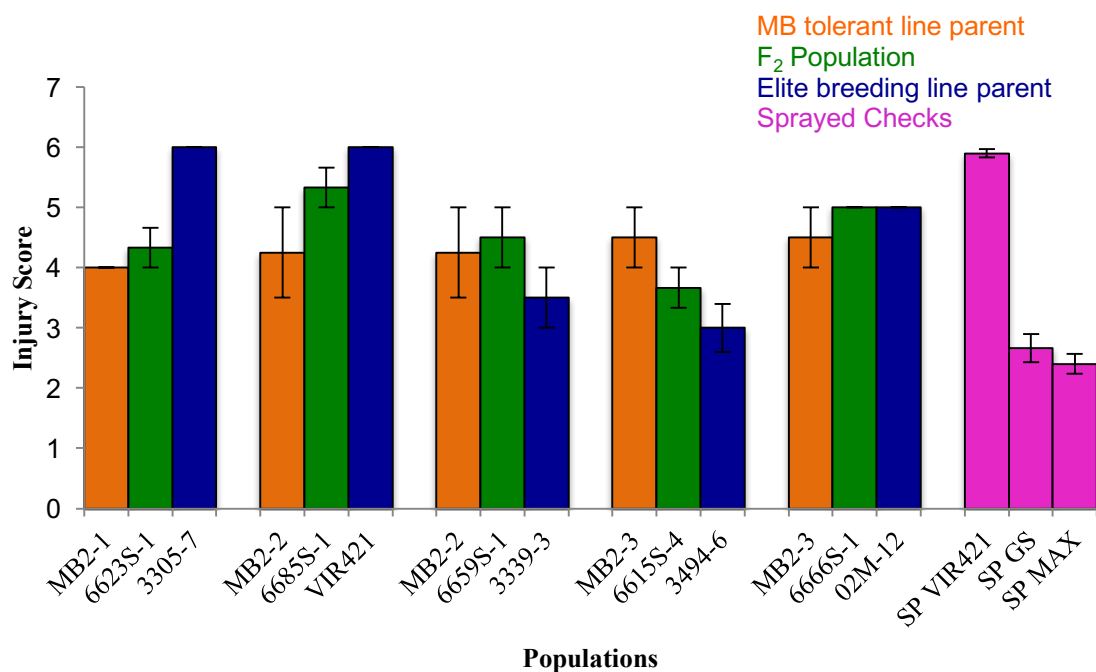


Figure 4.5 Injury score of MB-2 F₂ populations and parents 1 week after MB herbicide application. VIR 421 was the most susceptible towards MB.

Days to flower data had relatively minor variation. Elite breeding lines and the checks flowered a few days earlier compared to the MB lines and the F₂ segregating populations (Figure 4.6 and 4.7), but overall MB had a minor effect on initial flowering date.

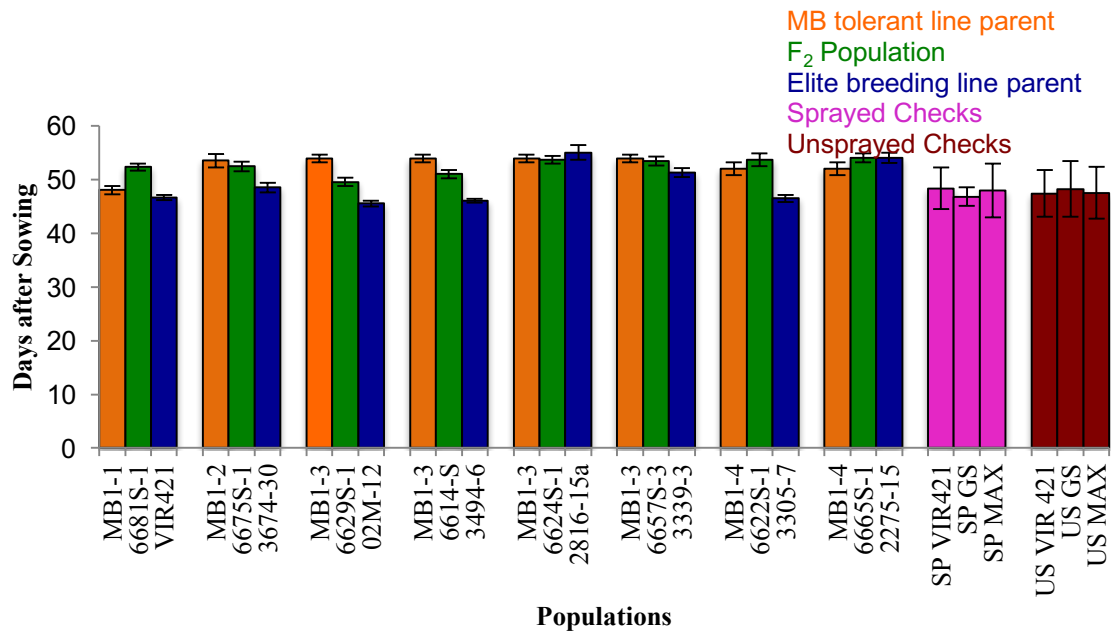


Figure 4.6 Days after sowing to flowering for MB-1 F₂ populations, MB tolerant parental lines and elite breeding lines. MB had little to no effect on initial flowering dates.

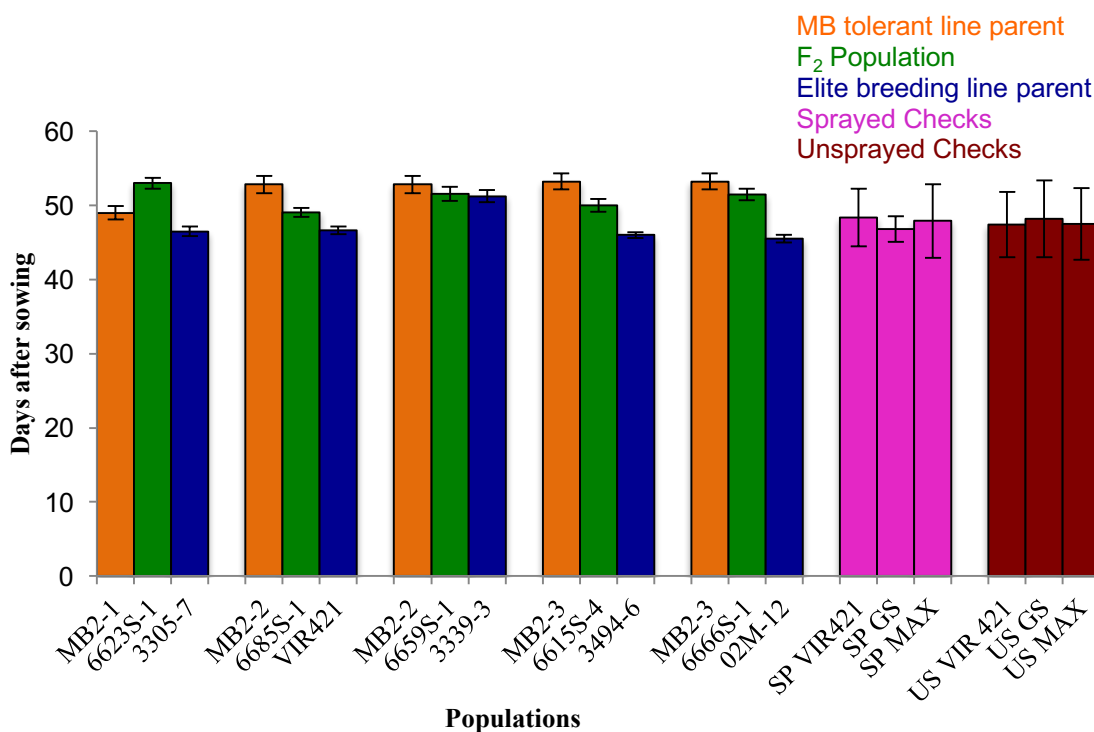


Figure 4.7 Days after sowing to flowering for MB-2 F₂ populations and parents, MB tolerant parental lines and elite breeding lines. MB had little to no effect on initial flowering dates.

Harvest index was affected by MB application to parents (MB tolerant lines and elite breeding lines) and the segregating F₂ populations (Figures 4.8 and 4.9). The harvest index was higher for all MB tolerant parental lines and F₂ populations compared to the elite breeding lines, except for the F₂ population 6629S-1. The harvest index of MB-1 crosses (Figure 4.8) was greater than that of segregating populations involving MB-2 (Figure 4.9). The harvest index for segregating populations developed from crosses with MB1-3 and MB1-4 parents was similar to that of the unsprayed checks, whereas most of the MB-2 populations had reduced harvest index compared to the checks.

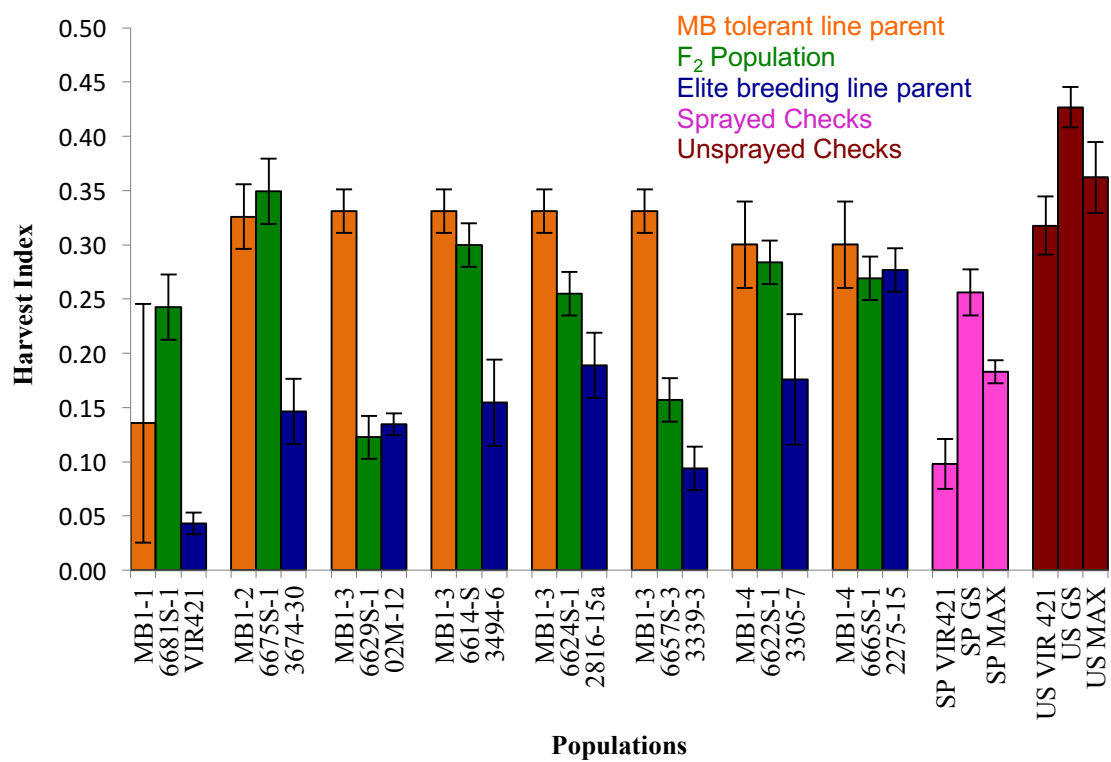


Figure 4.8 Harvest index of MB-1 F₂ populations, MB parental lines and elite breeding lines. MB parental lines and F₂ populations had larger harvest indices than elite breeding lines and sprayed checks.

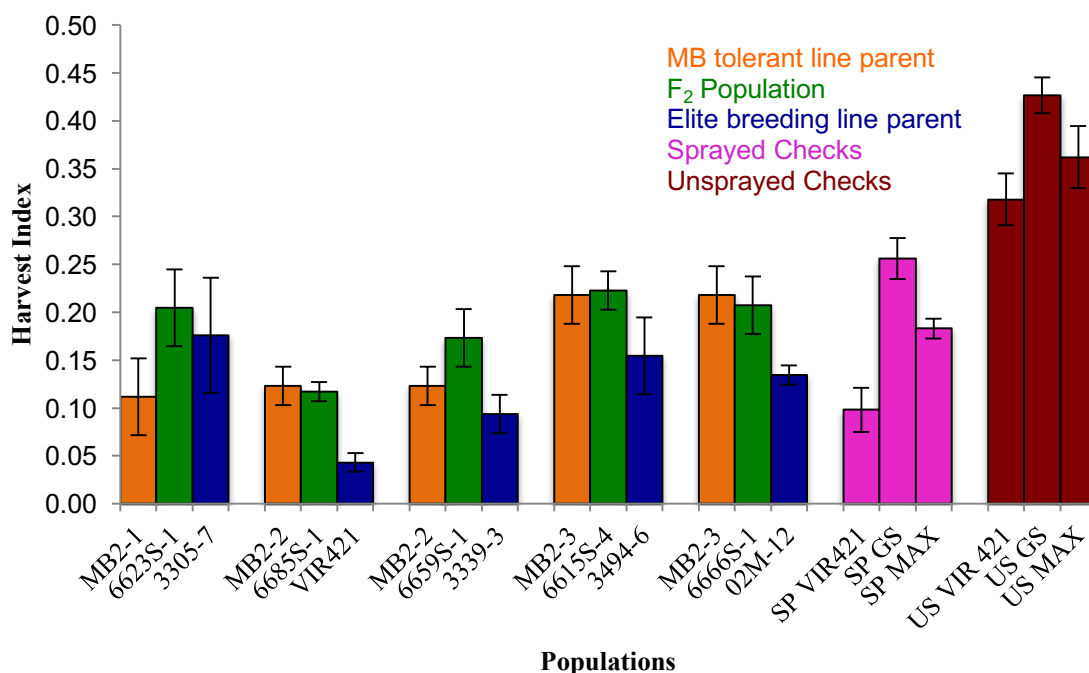


Figure 4.9 Harvest Index of MB-2 F₂ populations MB parental lines and elite breeding lines. MB parental lines and F₂ populations had larger harvest indices than elite breeding lines and sprayed checks.

4.1.2 DISCUSSION

In the summer of 2013, it was found that MB injury caused stand thinning, and the data likely reflected death of susceptible plants. The plants that did not die were severely damaged and regrew, indicating that there may be evidence of metabolic tolerance. This could explain the large difference in harvest indices of MB tolerant lines compared to non-elite breeding lines. Plausible genetic interpretations can be developed based on the hypothesis that there could be a major gene conferring tolerance in some crosses, and additive effects in others. CDC Greenstar and 3339-3 are the same cultivar, but the differences observed could be due to a different seed source, or an edge effect, because CDC Greenstar was always positioned in the check strips on the outer edge of

the plot strips. Also, flowering date did not appear to be affected by MB application. This is different than the response to imidazolinone susceptible lentil, for which flowering date is delayed with imidazolinone application (Chant 2004).

In the summer of 2014, field experiments on MB tolerance were continued from 2013 field experiments to screen selected genotypes, and to help determine the inheritance of MB improved tolerance. Follow up field studies were conducted to assess the reactions to MB application of sprayed (Saskatoon) and unsprayed (grown in New Zealand) subsets of the same populations of F₂-derived F₃ rows. A subset of the MB-1 lines was further investigated based on preliminary evidence of higher MB tolerance. The plants were sprayed with MB at the 3x rate. The intention was to gather data on injury and biomass from the field experiments. However, on July 11, 2014, it was evident that the expected tolerance was not sufficient for plants to survive a 3x MB application. This is most likely due to the wetter spring conditions, which allowed the MB to reach the root system at high concentrations in a short time-period. These populations are no longer being screened for MB tolerance.

However, the failure of these experiments showed that MB is a very unpredictable herbicide, and responds in a highly variable manner in response to specific environments. Variation may be due to both soil type, organic matter content, pH, and rainfall pattern. This is likely why the use of MB on lentil crops has decreased since the introduction of imidazolinone herbicide tolerance. The unpredictable results and false positives in the field experiments made it apparent that a more reliable indoor protocol was needed (Appendix 1). This would provide a more predictable tool for controlling the injury level in lentil, which would greatly assist with screening for MB tolerance. Also, new genetic sources of lentil with improved MB tolerance would be desirable, as the initial selected

populations did not show reliably improved tolerance under field experiments conducted in 2013 and 2014, unlike observations in previous years.

4.2 EXPERIMENT 2: ASSESSING METRIBUZIN TOLERANCE IN A MUTAGENIZED POPULATION OF CDC REDBERRY LENTIL

4.2.1 RESULTS OF DOSE RESPONSE STUDIES

The dose response studies showed that increasing rates of MB application decreased the amount of biomass produced by the lentil plants (Figure 4.10). PMBR-1 and 7516as had a higher ED₅₀ than CDC Redberry, and PMBR-3 and 7525s had the lowest ED₅₀ (Figure 4.10). PMBR-3, which had the lowest ED₅₀ of the three PMBR parents, is one of the parents for the 7525s F₂ population, which also had the lowest ED₅₀ of the F₂ populations. (Table 4.1).

Table 4.1 Effective doses metribuzin applied to mutant parental lines (CDC Redberry, PMBR-1, PMBR-2, PMBR-3) and F₂ populations (7516as, 7525as, 7529s)

Parent Genotype	Estimate (g a. i. h ⁻¹)	Std. Error
CDC Redberry (Parent)	671	6.30E+01
PMBR-1 (Parent)	715	1.03E+02
PMBR-2 (Parent)	511	8.68E+01
PMBR-3 (Parent)	335	5.63E+01
7516as (PMBR2)	679	1.33E+02
7525as (PMBR3)	594	8.02E+01
7529s (PMBR1)	611	8.20E+01

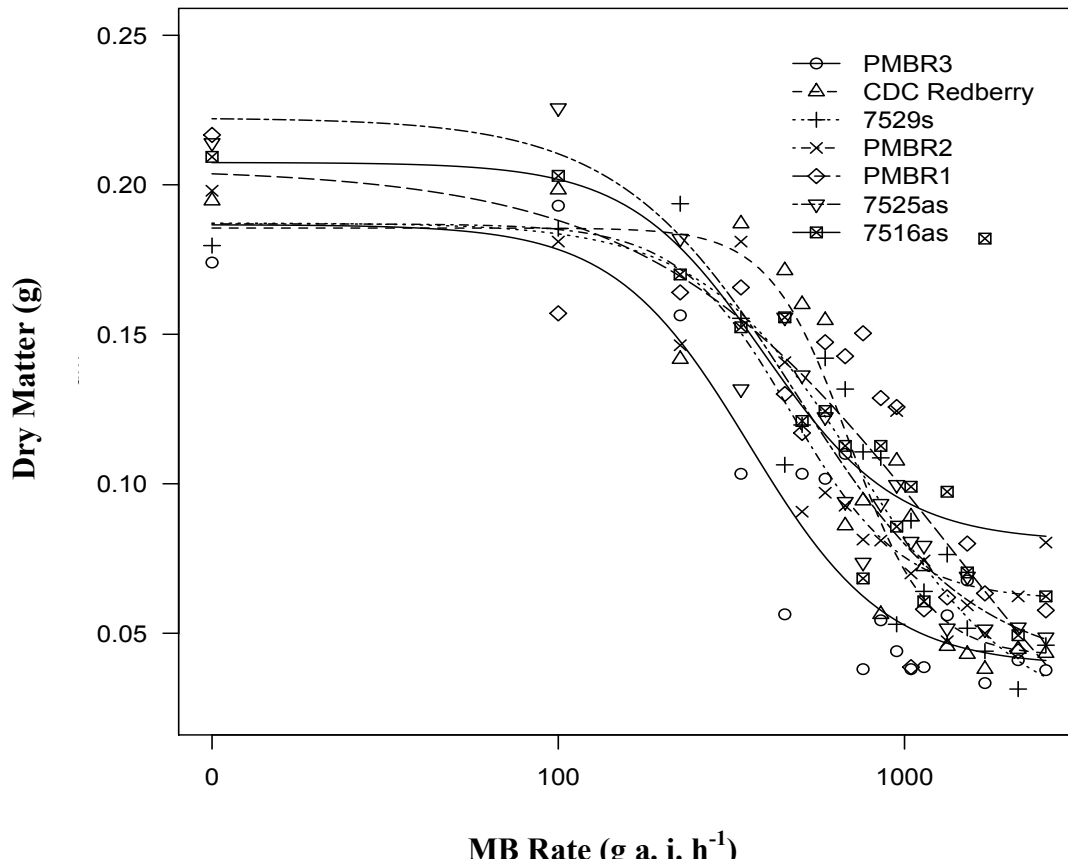


Figure 4.10 Dose response curve for mutant parents and F2 populations (7529s, 7525as, 7516as) showing that increased rates of metribuzin decrease the amount of plant biomass. The curves indicate lentil biomass, and are all significant ($P < 0.05$).

When the F₂ populations are compared to each other in the dose response study, the F₂ of 7529s had the highest ED₅₀ (750.42) and that of 7516as had the lowest ED₅₀ (390.76) of the three populations (Table 4.2, Figure 4.11). F₂ population 7529s also ranked the highest when the dose response study was based on visual rankings of percent injury from MB damage (Figure 4.12, Table 4.3). F₂ population 7516bs had a higher ED₅₀ (812) compared to 7525as (668). The MB tolerant parent of 7529s parent was PMBR-1, which also exhibited the greatest visual tolerance to metribuzin application.

Table 4.2 Effective doses of metribuzin when applied to three F2 populations of lentil

F2 Population	Estimate (g a.i. ha⁻¹)	Std. Error
7516as	390.76	117.47
7525as	487.55	139.87
7529s	750.42	239

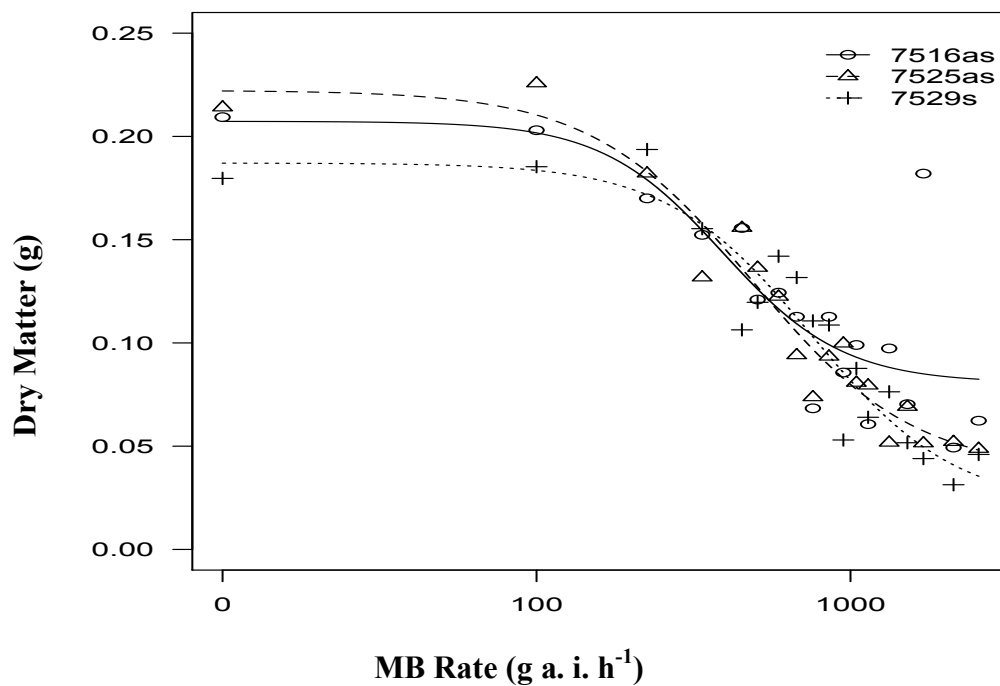


Figure 4.11 Dose response curves of 3 F2 populations of lentil showing that increased rates of metribuzin decreased the amount of plant biomass. The curves indicate lentil biomass. All are significant ($P < 0.05$). Lack of fit test is 0.9495.

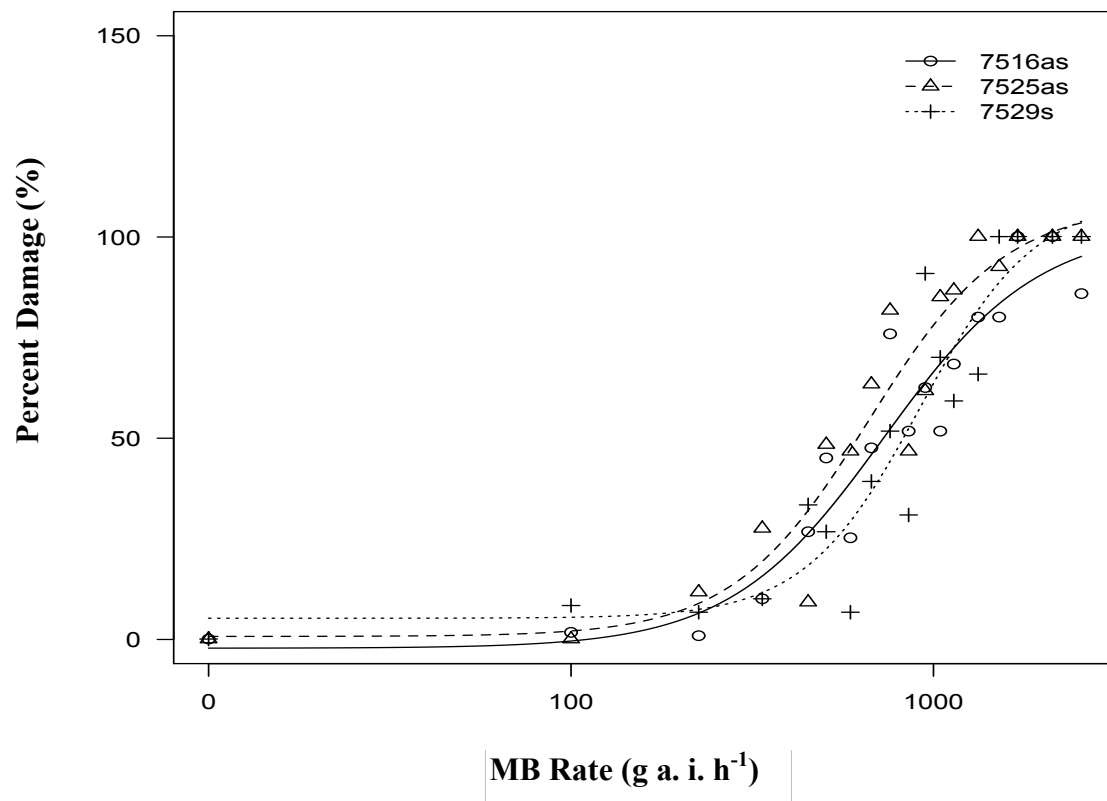


Figure 4.12 Dose response curve of 3 F2 populations of lentil showing that increased damage visual scores (percentt damage) correspond with higher rates of metribuzin application. The curves indicate visual damage score (% necrosis), and are all significant ($P < 0.05$). Lack of fit test is 0.9245.

Table 4.3 Effective doses of MB on F₂ populations based on visual damage (percent damaged)

F ₂ Population	Estimate (g a. i. ha ⁻¹)	Std. Error
7516as	812	1.15E+02
7525as	668	8.33E+01
7529s	872	96.2+01

4.2.2 F₂ GENETIC STUDIES

An F₂ genetic study of the response to MB was conducted in December 2015 on the mutant CDC Redberry population 7529s. The parents of the cross were PMBR-1 x CDC Redberry. The plant response was categorized as either dead or alive. A Chi-square was used to test the hypothesis that the 7529s F₂ population showed tolerance to MB at 800 g a.i. ha⁻¹ of 3 susceptible: 1 resistant. The 3:1 ratio was used to be tested based on visual observations from initial studies that appeared to be 3:1. The initial analysis of the genetic study showed that only 1/16 of the 7529s population had tolerance to MB at 800 g a.i. ha⁻¹, and the 3:1 ratio as hypothesized was rejected ($\chi^2(1, N=96)=0.19$, $p<0.05$). The F₂ plants that did survive had some damaged leaves. The parental line PMBR-1 also had both dead and live plants after MB application, indicating that segregation of MB tolerance could still be occurring in the progeny of the parental line. However, the previous dose response studies indicate that PMBR-1 and 7529s had increased tolerance to MB, and should be considered as candidate parental sources in the herbicide tolerance breeding program.

4.2.3 DISCUSSION

During the project, a mutagenized CDC Redberry population was used to identify a wider range of genetic variation for MB tolerance. This would create the opportunity to

explore new sources of improved tolerance. Several M₃ populations were screened at the 10x rate of MB, and three plants with putative MB tolerance were selected, crossed and screened indoors using dose response and genetic studies. The dose response study was carried out to rank the mutant parents and progeny with the highest ED₅₀. Based on the analysis, the genotype PMBR-1 and 7529s and 7516as are parental sources that should be used in MB tolerance breeding program. The origin of PMBR-1 remains unknown, and it is unlikely that is a mutant derived from CDC Redberry. It is a large green lentil, similar to CDC Greenstar, and SP1333, a genotype that was screened by Larn McMurray that showed increased MB tolerance. However, when comparing the standard errors, it is difficult to determine if there is a real difference between the ED₅₀, as the standard errors overlapped. The large standard errors are likely because the populations are still segregating for MB tolerance.

An initial genetic study was conducted on 100 F₂ plants of cross 7529s to determine the genetic inheritance of MB tolerance. It was hypothesized that there would be a 3:1 ratio of susceptible to tolerant plants, but the study suggested at 15:1 ratio exists. This is similar to the results of studies in MB tolerance in lupin (Si et al. 2009), indicating that double recessive inheritance could be a possible mode of inheritance. Another genetic study with lentil should be conducted to further explore the basis for inheritance of this source of MB tolerance.

4.3 EXPERIMENT 3: ASSESSING METRIBUZING TOLERANCE IN COMMERCIAL LENTIL CULTIVARS

4.3.1 FALL 2014 OUTDOOR MB SCREENING

Significant differences for MB tolerance as measured by biomass reduction was observed among the commercial genotypes and elite breeding lines of lentil at the 4x application rate of MB (Figure 4.13). The biomass reduction from the 4x rate of MB ranged from 40% to 10% (Figure 4.13) CDC Greenstar and Eston had the lowest biomass reduction, whereas VIR 421, CDC Greenland and 3592-13 (CDC Kermit) had the highest biomass reduction. When genotypes were grouped by market class, no differences were found in biomass reductions between market classes (Figure 4.14).

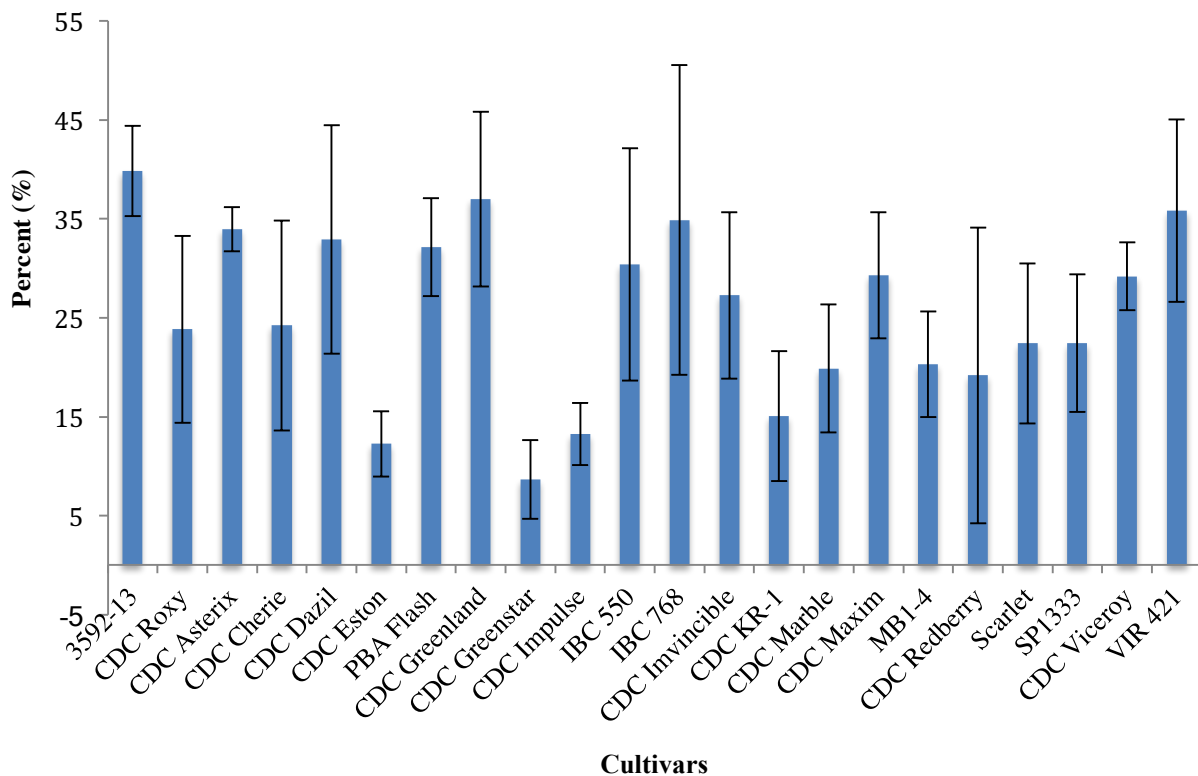


Figure 4.13 Mean percent biomass reduction of commercial cultivars and elite breeding lines sprayed with metribuzin at 4x (824 g a.i. h⁻¹) in Fall 2014

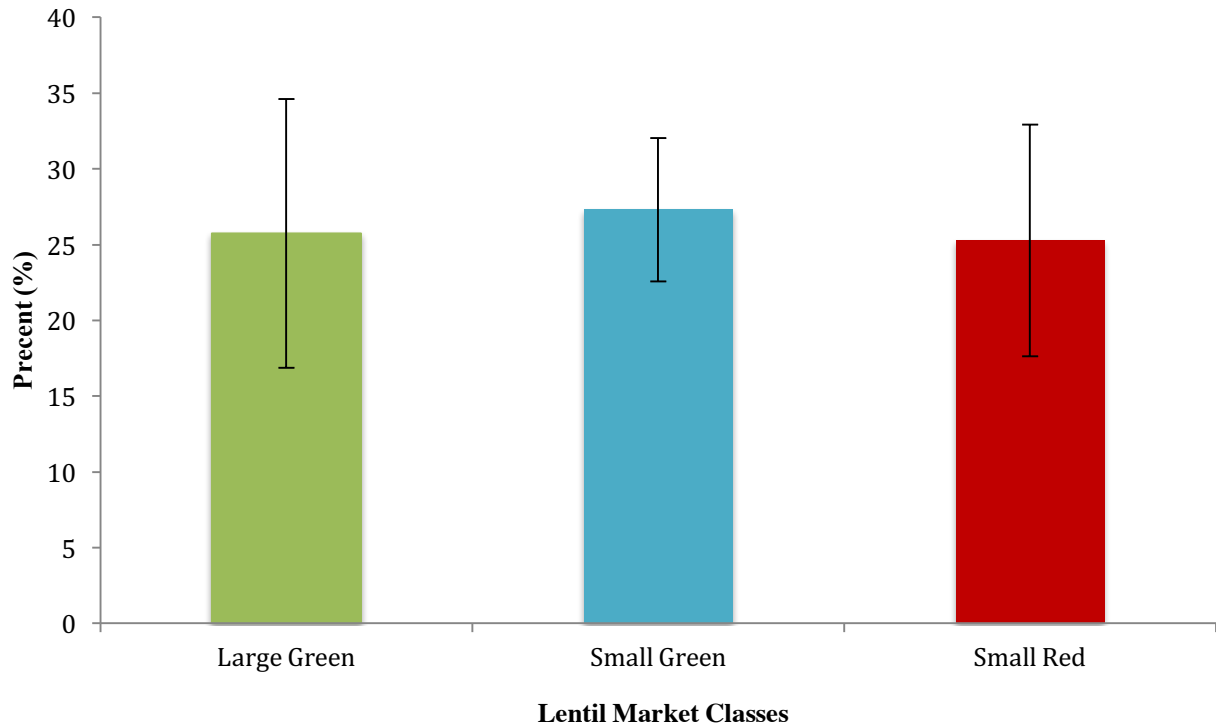


Figure 4.14 Mean percent biomass reduction by market classes for lentil cultivars sprayed with metribuzin at 4x (824 g a.i. ha⁻¹) in fall 2014

4.3.2 OUTLOOK VS. SPG SCREENING LOCATIONS

Significant variation in MB tolerance between rates and market classes were observed at Outlook and SPG (Table 4.4). Despite the difference in soil type, no difference was observed between the two sites (Table 4.4). This is surprising because there was a higher rating for visual damage at the Outlook site compared to the SPG site (Figure 4.15). The lack of difference between sites may have occurred because at SPG there was a sprayer error caused by a burst spray bottle, and part of the 4x spray zone at SPG received an extremely high amount of herbicide. Sampling from this area was avoided. The SPG site had high amounts of wheat straw residue from the previous wheat

crop on the soil surface. This may have interfered with herbicide transfer to the root zone therefore data and graphs will only be presented for the results from the Outlook location when comparing indoor and outdoor results.

Table 4.4 Analysis of variance for the effect of MB rate (0.5x, 1x, 2x, 4x=824 g a.i. ha⁻¹) on biomass reduction of market classes for lentil cultivars at Outlook and SPG

Effect	Degrees of Freedom	F Value	Pr>F
Experiment	1	0.75	0.3866
Rep	2	0.06	0.9402
Market class	5	11.07	<.0001
Rate	3	396.98	<.0001
Rate*Market class	15	0.76	0.7198

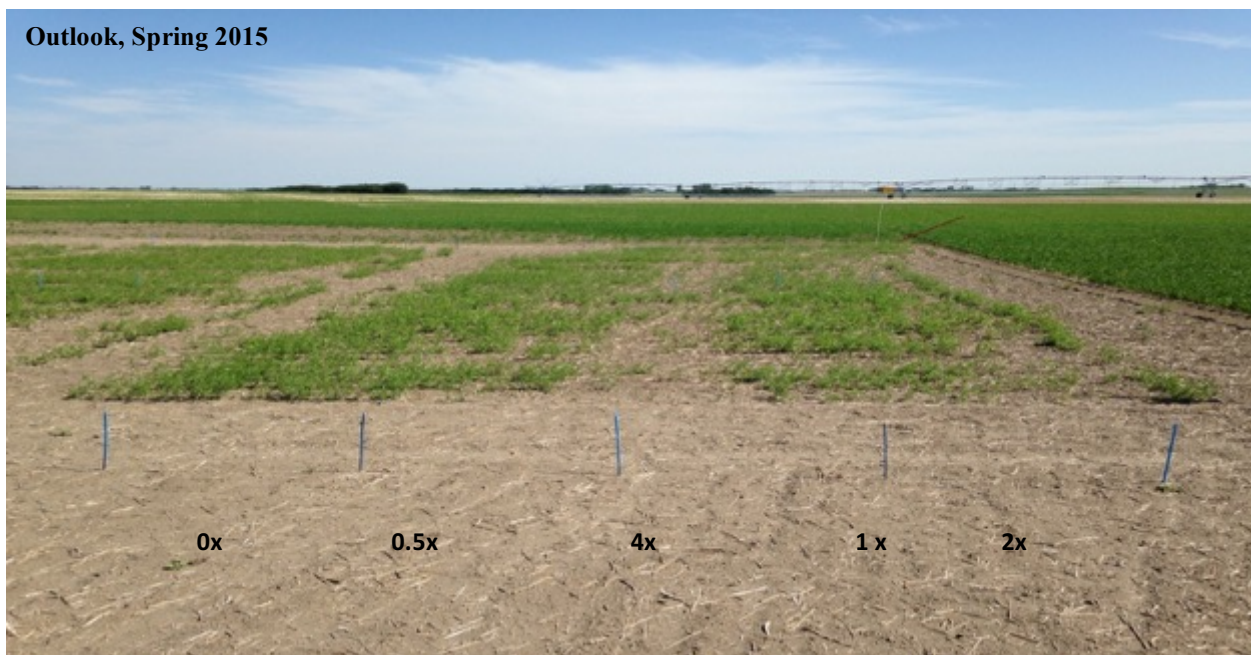


Figure 4.15 Sites used in in 2015 for screening metribuzin tolerance of lentil commercial cultivars and elite breeding lines. Both Outlook and SPG had irrigation set up. SPG had excessive straw residue.

4.3.3 INDOOR VS. OUTDOOR STUDIES

Both field and indoor experimental results (Figures 4.16, through 4.21) illustrated that variability exists among lentil genotypes for reaction towards MB applications at 0.5x, 1x, 2x and 4x. For all genotypes, the 4x application resulted in largest amount of biomass reduction. The Australian cultivar PBA Flash (small red market class) and VIR 421 (small green market class) are the two known highly susceptible lines tested, and both had the greatest biomass reduction within their respective market classes (Figure 4.16 and 4.17, 4.19, 4.20). Also noted is that IBC 768 is an imidazolinone tolerant backcross of CDC Greenstar and is a backcross of CDC Greenstar and it had greater biomass reduction compared to CDC Greenstar. The reduction in MB tolerance of IBC 768 could be due to inconsistent use of MB herbicide in the lentil breeding program in recent years. This intermittent pattern of MB screening in the lentil breeding programs over the past 10 years may have led to genetic drift resulting in susceptibility application to MB in some commercial cultivars.

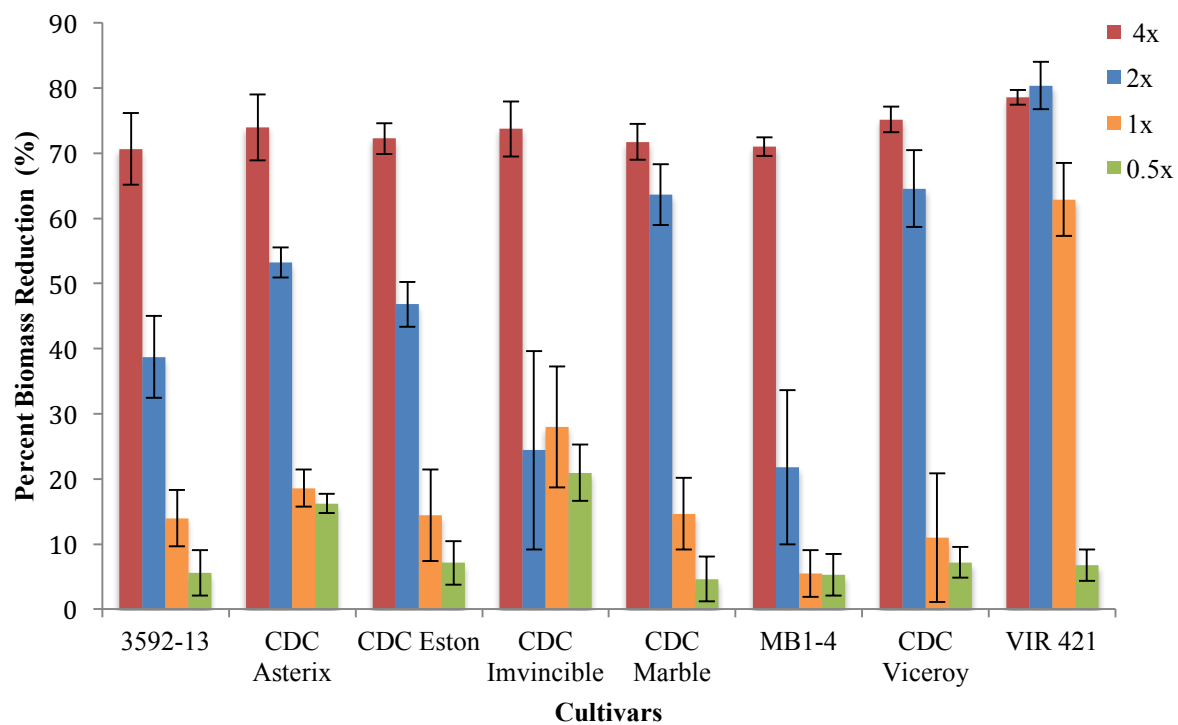


Figure 4.16 Mean percent biomass reduction of small green lentil commercial cultivars and elite breeding lines sprayed with MB in indoor conditions. 1x = 206 g a.i. ha⁻¹

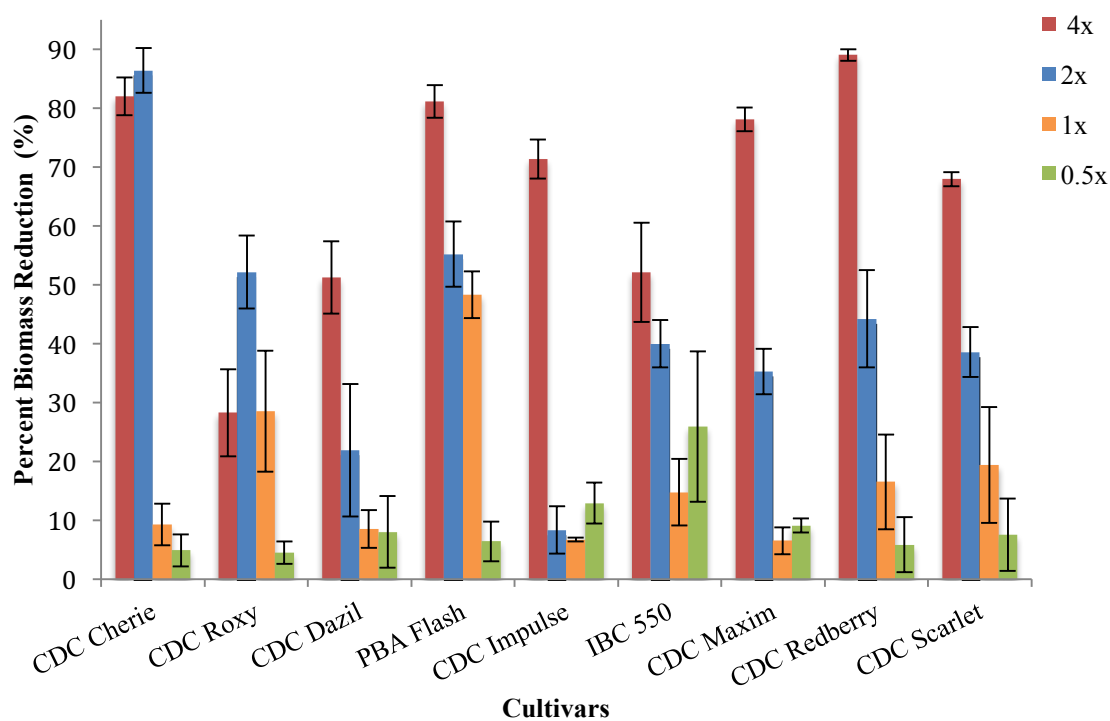


Figure 4.17 Mean percent biomass reduction of small red lentil commercial cultivars and elite breeding lines sprayed with MB in indoor conditions. 1x = 206 g a.i. ha⁻¹. CDC Roxy is not being used for comparison, as the raw data was likely incorrectly recorded.

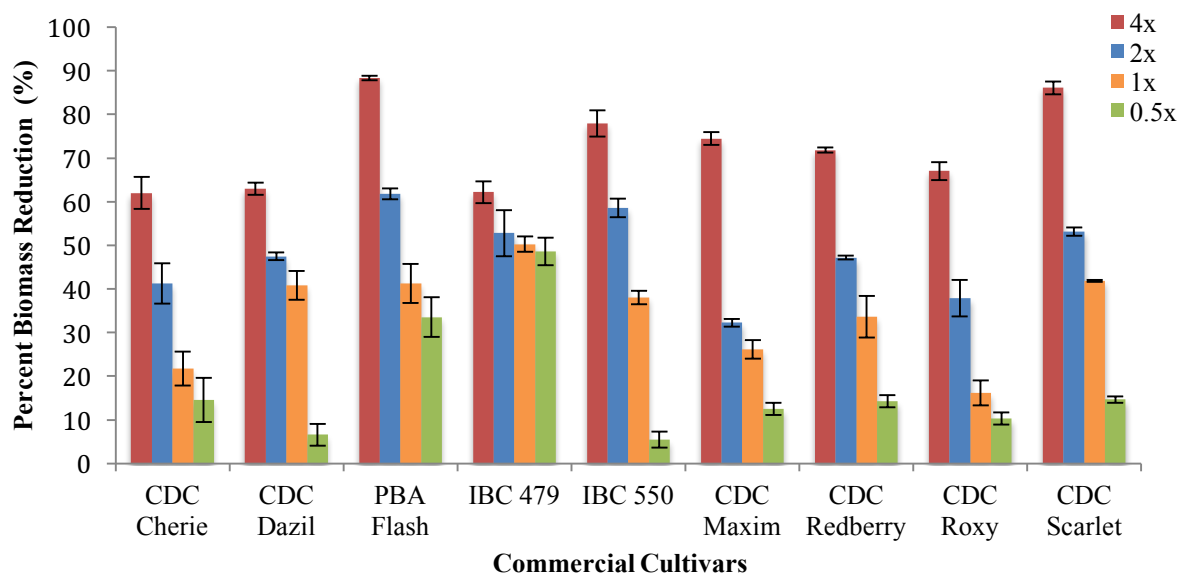


Figure 4.18 Mean percent biomass reduction of small red lentil commercial cultivars and elite breeding lines sprayed with MB in outdoor conditions. 1x = 206 g a.i. ha⁻¹

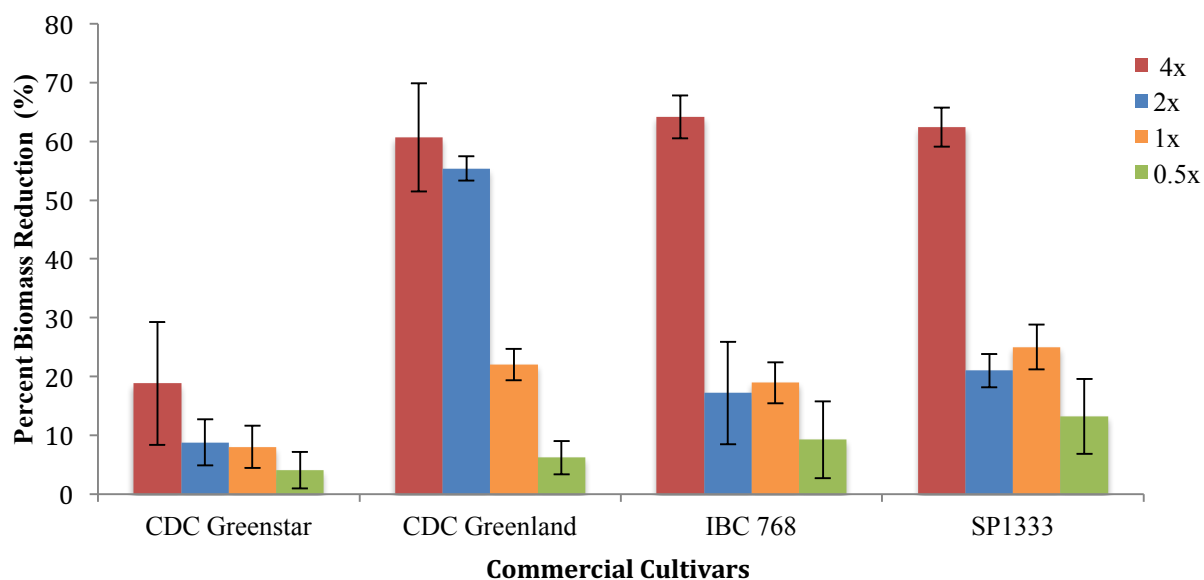


Figure 4.19. Mean percent biomass reduction of large green lentil commercial cultivars and elite breeding lines sprayed with MB in indoor conditions. 1x = 206 g a.i. ha⁻¹

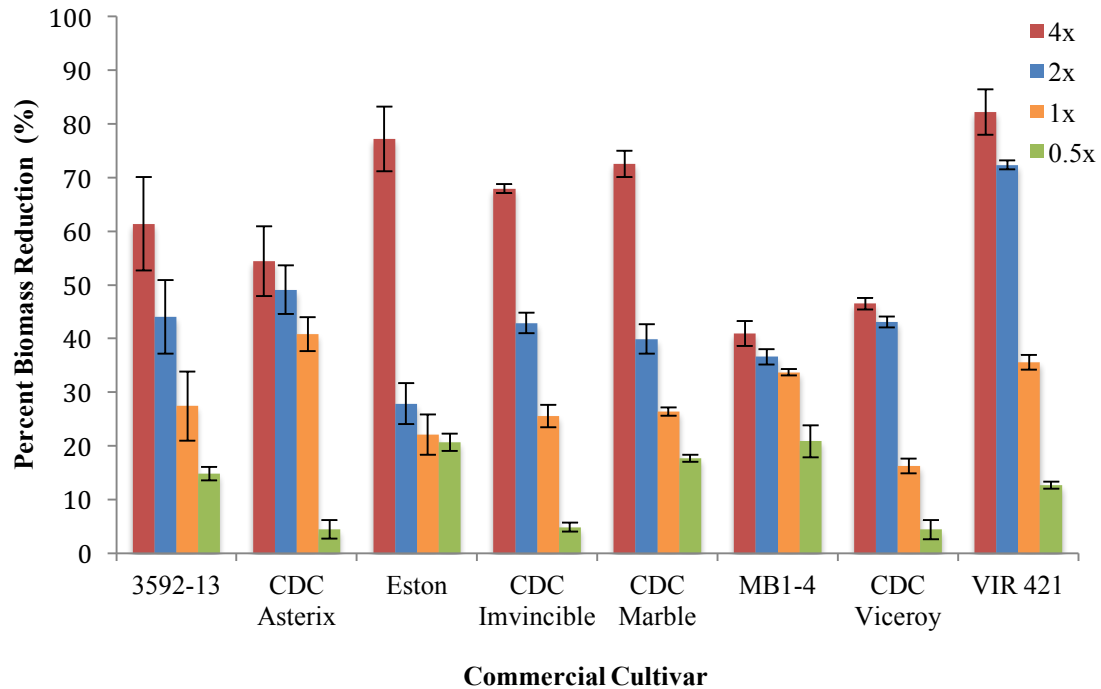


Figure 4.20 Mean percent biomass reduction of small green lentil commercial cultivars and elite breeding lines sprayed with MB in outdoor conditions. 1x = 206 g a.i. ha⁻¹

Variation in MB tolerance was also observed when commercial cultivars or elite breeding lines were grouped by market classes (Figure 4.22). When genotypes were grouped as market classes in the indoor experiments, the large green market class had the least amount of biomass reduction at the 4x MB application, which is confirmed by the field experiments (Figures 4.23, Figure 4.24). At the 2x rate, the large green market class has a different response to MB compared to the small red market class, but not in comparison to the small green market class (Figure 4.23, Figure 4.24). At 0.5x and 1x rates of MB, there were no differences among market classes in terms of biomass reductions. Under both indoor and field conditions, there was a significant difference between rates ($P < 0.001$) in terms of biomass reductions, and a significant difference

between market classes ($P < 0.001$). There was no interaction between MB application and market class (Indoor: $P = 0.2903$, Field: $P = 0.5068$) (Table 4.5, Table 4.6).

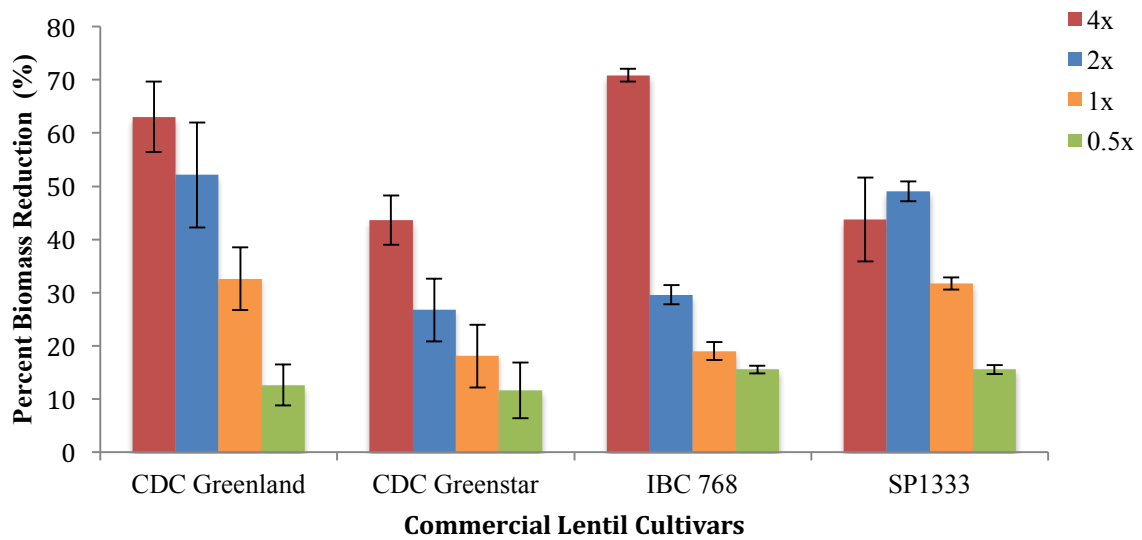


Figure 4.21 Mean percent biomass reduction of large green lentil commercial cultivars and elite breeding lines sprayed with MB in outdoor conditions. 1x = 206 g a.i. ha⁻¹

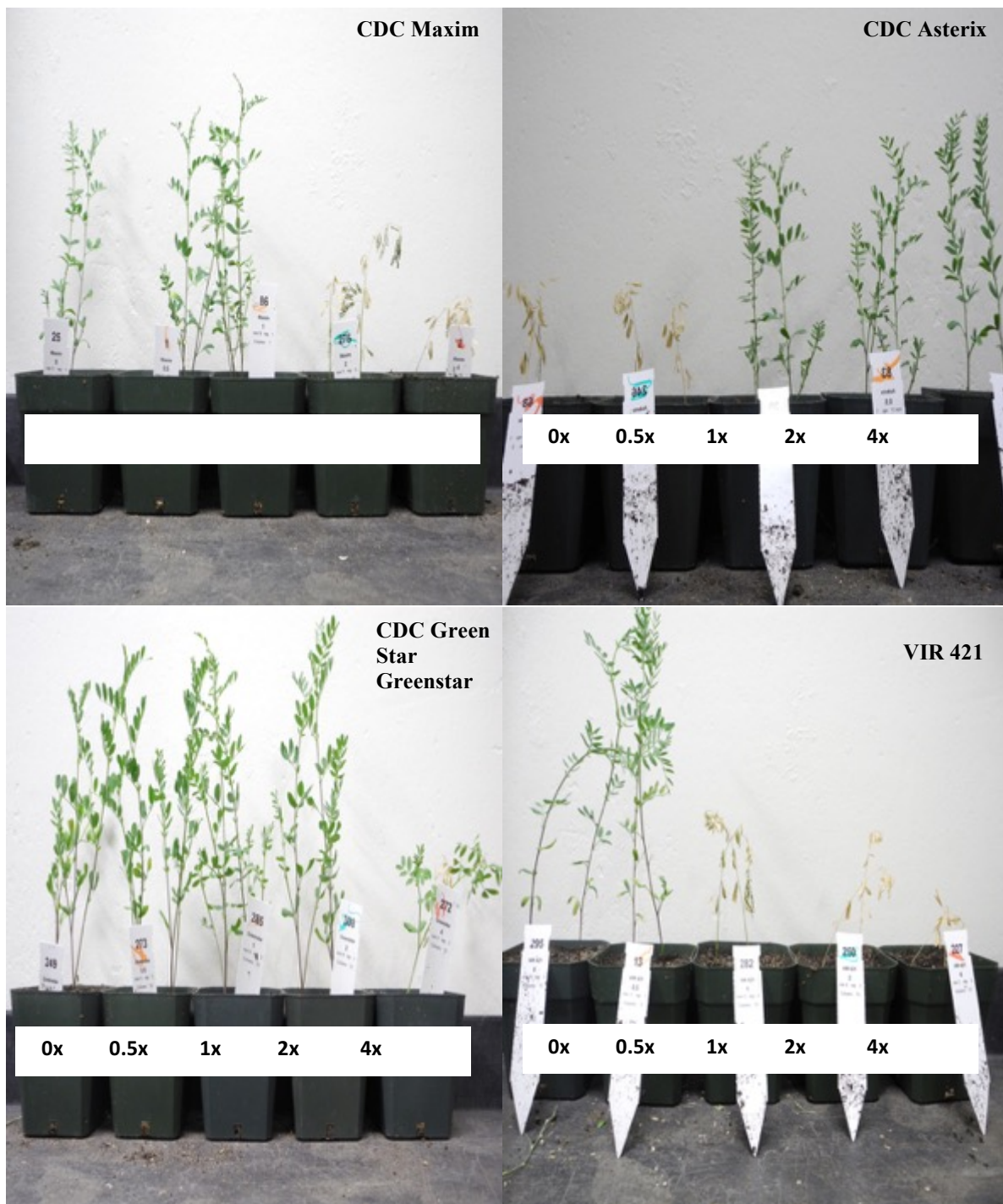


Figure 4.22. Photographs of typical injury observed in the indoor study of MB rate application involving different lentil market classes. Levels of plant injury are shown for CDC Maxim (small red), CDC Asterix (small green), CDC Greenstar (large green) and VIR 421

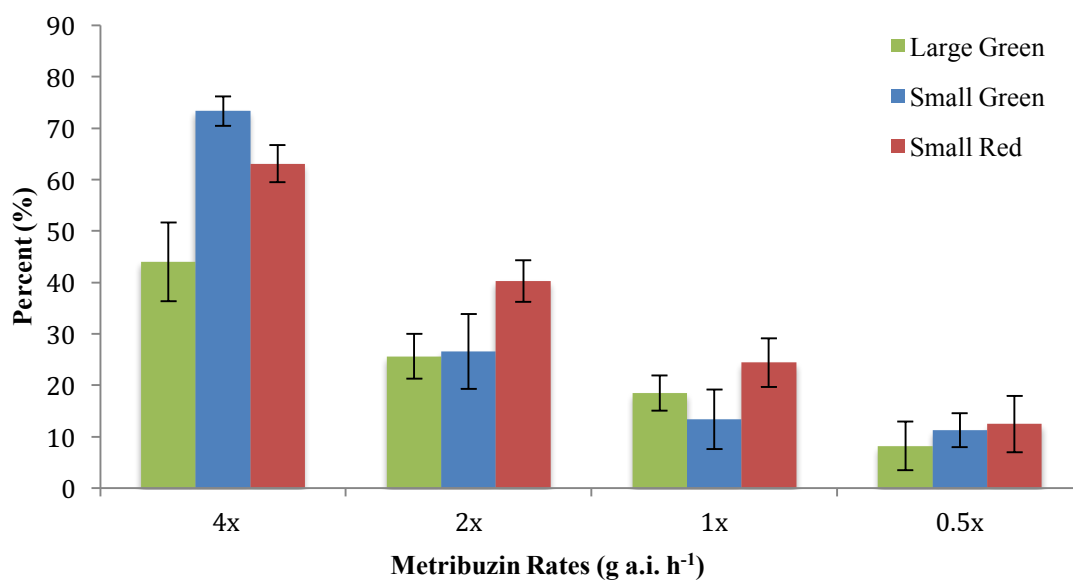


Figure 4.23. Mean percentage of biomass reduction of lentil market classes sprayed with four rates of MB under field conditions (1x=206 g a.i. ha⁻¹)

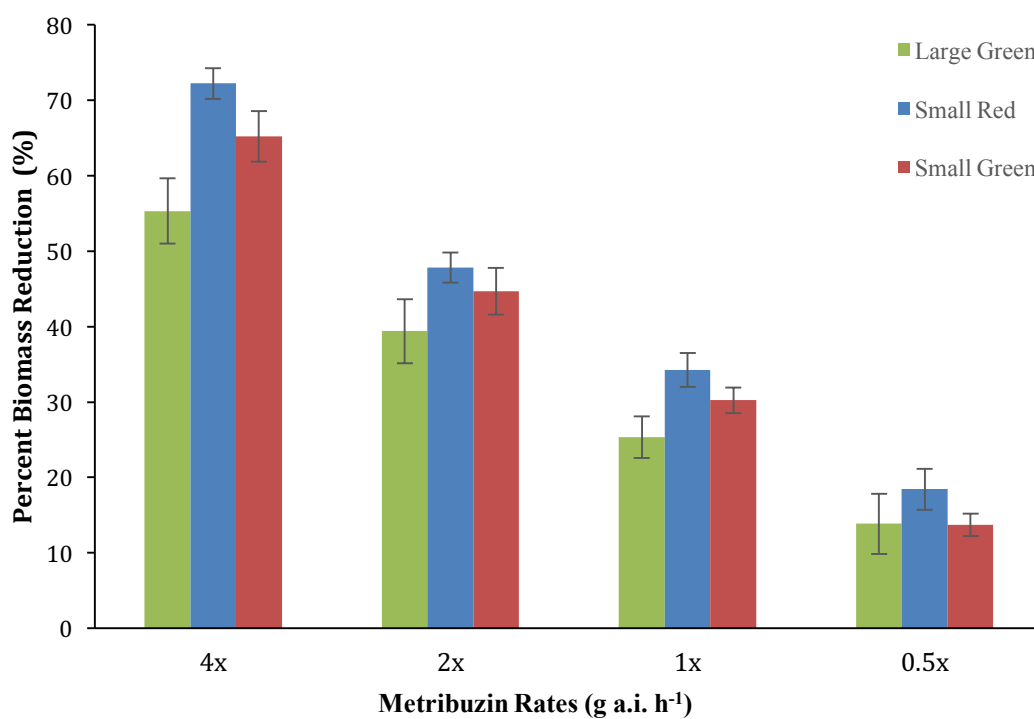


Figure 4.24. Mean percentage of biomass reduction of lentil market classes sprayed with four rates of MB in indoor conditions (1x=206 g a.i. ha⁻¹)

Table 4.5. Analysis of variance for the effect of MB rate on biomass reduction in field conditions of lentil market classes.

Effect	Degrees of Freedom	F Value	Pr>F
Market	5	116.98	<.0001
Rate	3	9.58	<.0001
Rate*Market	15	0.95	0.5068

Table 4.6. Analysis of variance for the effect of metribuzin rate on biomass in indoor conditions of lentil market classes.

Effect	Degrees of Freedom	F Value	Pr>F
Market	5	82.98	<.0001
Rate	3	5.80	<.0001
Rate*Market	15	1.18	0.2903

In both indoor and field experiments, ratings for genotypes had large standard errors. This occurred when one plant of the same genotype within a pot had signs of chlorosis or necrosis, while the other plant would be completely healthy (Figure 4.25).



Figure 4.25. This photo shows two plants within the same pot of the same spray treatment and genotype, but displaying different symptoms in response to metribuzin application. This could be due to heterogeneity of metribuzin tolerance within genotypes.

4.3.4 DISCUSSION

MB was intermittently used for supplemental weed control in the lentil breeding program since 2010, and was only used in specific environments where conditions led excessive weed flushes, or when imadazolinone herbicides were not fall applied. Since the use of MB was no longer generally used in the lentil, it was of interest to gain insight into the historical services of CDC cultivars and elite breeding lines. This could provide information on new sources of MB tolerant genetic material, and provided comparisons of cultivars in response to consistent MB applications.

The initial experiment was conducted outdoors in fall of 2014. The only replicaton of the experiment that was harvested for assesment of biomass was the one which MB was applied at 4x rate, the only replication that showed visual damage. The lack of differences in biomass reduction could be due to the fall growing conditions. The experiment was seeded late at the time of onset of cooler, shorter days. This could be the reason for slower growth, and therefore the MB uptake through the root system was reduced. Slower growth of the lentil plants likely means there is less MB uptake, hence less damage from MB. The cooler fall temperatures experienced during this experiment may have hindered the effectiveness and uptake of herbicide, therefore resulting in less biomass reduction. However, variation was observed within seed size classes. Eston, a small-seeded green lentil, had less injury than other lentil genotypes with similar seed size. Eston was the first small-seeded lentil cultivar released in Canada (Slinkard 1981). It was released around the same time that MB was registered, so during the pure line selection process it was likely subjected to MB screening during the development of a cultivar. In all MB screening experiments, CDC Greenstar consistently showed less MB injury compared to all of the other commercial cultivars and elite breeding lines. MB

application to CDC Greenstar can be recommended for 1x rates in commercial production without fear of crop injury.

One of the major challenges for screening for MB tolerance under field conditions is identifying a location with irrigation and with soil characteristics that will not interfere with MB uptake and thereby reduce false positive results. We minimized soil organic matter material in the phytotron experiments by adding sand to the soil medium, combined with use of a strict watering regime (Appendix 1). To achieve similar outdoor results, Outlook SK was selected as a location for screening for MB tolerance of commercial cultivars. Unlike the SPG site, soils in the Outlook area have a higher percentage of sand and there is access to reliable irrigation.

Another objective was to develop an indoor protocol that has comparable results to outdoor conditions. The two major challenges of indoor conditions were to optimize plant health, while minimizing organic matter in the soil medium to obtain consistent MB damage. This was achieved with the assistance of Larn McMurray after a series of trials and errors. The protocol described in Appendix 1 was developed from these experiments, and it is now used as the current standard of practice for screening lentil for MB tolerance in the UofS phytotron.

The MB protocol was successful as it demonstrated similar results under both indoor and outdoor conditions. Both experiments showed that the large-seeded lentil market classes had the most tolerance to MB at 4x, and that there were significant differences between market classes and application rates ($P < 0.001$). This simplifies the procedure for screening for MB tolerance indoors in controlled environments. An important factor for successful screening MB tolerance in lentil is ensuring that MB reaches the root system of the plant, where optimal damage occurs. Sometimes,

however, within the same pot, completely dead plants and completely healthy plants are observed. This could occur if lentil genotypes are heterogeneous for MB tolerance, which is possible because the lentil breeding program is based on the F₂ family derived method. We might expect that there is residual heterogeneity for reaction to MB injury, and that tolerance for MB could be maintained in the population.

To achieve optimal MB damage in screening for herbicide tolerance, it is essential for MB to reach the root system. False positives can occur if the MB is bound in organic matter in the soil medium, thereby preventing root uptake of MB. Profile® Greens Grade™ is a profile porous ceramic inorganic soil mixture which was tested to determine if it would be an appropriate medium for growing lentil seedlings for the purpose of screening them for MB tolerance (Appendix 2). Initial experiments showed that it was difficult to keep the seeds from popping out of the soil media. Use of this medium caused excessive branching in the root system of lentil, and the plants appeared to be quite healthy overall. Profile® Greens Grade™ may be a good replacement for sand in the soil media. Sources of sand are completely unregulated, while Profile® Greens Grade™ is produced by a more predictable manufacturing process.

CHAPTER 5

5. GENERAL DISCUSSION

The initial objective of this research project was to characterize the genetic inheritance of MB tolerance in lentil F₂ populations derived from crosses between susceptible and tolerant parents. After 2013, it was evident that MB damage occurred, and that the MB-1 populations outperformed the MB-2 populations, in that they had more tolerance to MB at 3x. There is also evidence of improved MB tolerance because of the variability seen in parental lines and the different levels of response in progeny. However, after the summer of 2014, when all the populations were severely damaged by MB applications in the field, it was quickly realized that there needed to be more exploratory research to determine what would be the most appropriate method to screen lentil populations with MB, both indoors and outdoors.

It was quickly understood that there is a large genotype by environment interaction, and that amount of organic matter has an impact on MB damage on lentil plants. A screening method was developed that allows screening based on MB uptake by the lentil root system, as that is when the most MB damage will occur. Also, new genetic material with increased MB tolerance was identified and brought into the breeding program, as the initial genetic sources were not tolerant as originally observed in similar field environments but under different climatic conditions. Genetic variation towards MB tolerance was identified in commercial cultivars, with CDC Greenstar showing at least 1x MB tolerance.

From the mutagenized populations, we found that PMBR-1 and its progeny 7529s showed improved tolerance towards MB, but the large standard errors indicate that

it could still be segregating towards MB tolerance. But, these populations should be incorporated into the breeding program as parental sources.

Overall, we know a lot more about improved methodology and predictability of screening for MB tolerance in lentil populations than we did when the project started. The effects of MB on lentil plants are unpredictable, causing of a pattern of weed control and subsequent inconsistent use in the lentil breeding program. MB has highly variable effects on lentil plants within and between environments. Observations from the past experiments show that organic matter interacts with MB, can decrease the efficacy of the herbicide. More studies should be done to determine the amount of organic matter that would cause an interaction with organic matter, as well if pH may also have an influence on MB efficacy.

For the lentil breeding program, it is essential to bring the MB herbicide in contact with the root system to create an environment where consistent MB damage will occur, as ultimate damage occurs when MB reaches the roots and is transported actively during growth. This can be achieved in controlled indoor conditions (Appendix 1), or in outdoor environments and conditions that provide sandy soils and ready access to irrigation. The sandy soil conditions are essential for screening F₂ populations, so that segregating populations can be damaged sufficiently by MB to allow selection of tolerant individuals while minimizing the number of false positives.

Breeding for MB tolerance requires both a quantitative and a metabolic approach. Germplasm development needs to be designed so that MB tolerance increases in frequency in each generation. It is likely that multiple genes are involved, and that recombination is needed to optimize the identification of superior lentil genotypes with improved MB tolerance.

LITERATURE CITED

- AAFC. 2007. Lentil Profile. Available at: <http://www4.agr.gc.ca/AAFC-AAC/display-afficher.do?id=1174596720488&lang=eng>
- Agarwal, S.K. 2009. Pesticide Pollution. APH Publishing Corporation. New Delhi: 67
- Basler, F. (1981). Weeds and their control. In Lentils. London. Commonwealth Agricultural Bureaux: 143-153.
- Banks, P.A., and E.L Robinson. 1982. The influence of straw mulch on the soil reception and persistence of metribuzin. Weed Science, 30(2): 164-168.
- Barulina, H. (1930). Lentils of the USSR and of Other Countries. 40th Supplement to the Bulletin of Applied Botany, Genetics and Plant Breeding: Leningrad: 265- 230 (English Summary).
- Bayer CropScience Inc. 2011. Herbicides. Sencor. Available at: <http://www.bayercropscience.ca/products/herbicides/sencor/>
- Beckie, H. 2012. Herbicide resistance update. Agronomy Update Conference: Integrated Pest Management (IPM). Alberta Agriculture and Rural Development.
- Beckie, H. and X. Reboud. 2009. Selecting for weed resistance: herbicide rotation and mixture. Weed Technology, 23: 363-370.
- Boerboom, C., and F. Young. (1995). Effect of postplant tillage and crop density on broadleaf weed control in dry pea (*Pisum sativum*) and lentil (*Lens culinaris*). Weed Technology, 9(1): 99-106.
- Brand, J., N. Yaduraju, B. Shivkaumar, and L. McMurray. 2007. Weed management. In Lentil: An Ancient Crop For Modern Times Springer, Dordrecht, the Netherlands: 159-172.
- CFIA. 2012. The Biology of *Lens culinaris* Medikus. Available at: <http://www.inspection.gc.ca/plants/plants-with-novel-traits/applicants/directive-94-08/biology-documents/lens-culinaris-medikus-lentil-/eng/1330978380871/1330978449837>
- Chant, S. R. 2004. Imidazolinone Tolerance in Lentil (*Lens culinaris* Medik). M.Sc. Thesis. University of Saskatchewan, Saskatoon, Canada.
- Ersine, V. 2009. Global Production, Supply and Demand. In The Lentil: Botany, Production and Uses. CABI Publishing: 4-12.

- Devine, M.D., and A. Shukla. 2000. Altered target sites as a mechanism of herbicide resistance. *Crop Protection*, 19(8-10): 881–889.
- Dekker, J.H., and S.O. Duke. (1995). Herbicide-resistant field crops. *Advances in Agronomy*, 54: 69-116.
- Fedoruk, L.K., and S.J. Shirtliffe. 2011. Herbicide choice and timing for weed control in imidazolinone-resistant lentil. *Weed Technology*, 25: 620-625.
- Global Crop Diversity Trust. 2008. Global strategy for the ex situ conservation of lentil (Lens Miller). Available at http://www.croptrust.org/documents/web/LensStrategy_FINAL_3Dec08.pdf
- Hall, L., H. Beckie, and T.M. Wolf. 1999. *How Herbicides Work: Biology to Application*. Alberta Agriculture and Rural Development, Edmonton, Alberta, 56-60.
- Heap, I. 2014. International survey of herbicide resistant weeds. Available at www.weedscience.org
- Ilicicco M., V. Viscos, S. Arena, A. Scaloni, D. Tupiano, M. Rocco M, D. Chiantante, and G.S. Scrippa. 2012. *Lens culinaris* Medik. seed proteome: analysis to identify landrace markers. *Plant Science*, 197:1-9.
- Koepki-Hill, R.M., G.R. Armel, K.W. Bradley, W.A. Bailey, H.P. Wilson, and T.E Hines. 2011. Evaluation of flufenacet plus metribuzin mixtures for control of Italian ryegrass in winter wheat. *Weed Technology*, 25:563-567.
- Ladizinsky, G. 1979. The origin of lentil and its wild genepool. *Euphytica*, 28(1): 179-187.
- McNeil, D.L., and M. Materne. 2007. Rhizobium management and nitrogen fixation. *In* *Lentil: An Ancient Crop For Modern Times*. Springer, Dordrecht :127-143.
- McVicar, R., P. McCall, C. Brenzil, S. Hartley, K. Panchuk, P. Mooleki, and D. Risula 2010. Lentil in Saskatchewan. Available at <http://www.agriculture.gov.sk.ca/Default.aspx?DN=c5993bcc-009f-4031-b936-c52c992b9e7d>. Accessed April 20, 2012
- Pan, G., P. Si, Q. Yu, J. Tu, and S. Powles. 2012. Non-target site mechanism of metribuzin tolerance in induced tolerant mutants of narrow-leafed lupin (*Lupinus angustifolius* L.). *Crop and Pasture Science*, 63: 452-458
- Profile®, 2015. Profile Porous Ceramic (PPC) Greens Grade. Available at: <http://www.profilevs.com/productpage/profile-porous-ceramic-ppc-greens-grade>
- SAS Institute Inc. 2012. SAS/STAT User's Guide 9.3. Cary, NC, USA: SAS Institute. Inc.

- Saari, L.L., J.C. Cotterman, and D.C. Thill. 1994. Resistance to acetolactate synthase-inhibiting herbicides. *In* Herbicide Resistance in Plants: Biology and Biochemistry. Boca Raton, Florida, USA: Lewis Publishers: 83–139
- Saskatchewan Ministry of Agriculture. 2010. Lentils in Saskatchewan. Available at <http://www.agriculture.gov.sk.ca/Default.aspx?DN=c5993bcc-009f-4031-b936-c52c992b9e7d>
- Saskatchewan Ministry of Agriculture. 2013. 2011 Specialty Crop Report. Available at: [http://www.agriculture.gov.sk.ca/Default.aspx?DN=1ca\)a615-f827-47ef-983e-becff77ba4f1](http://www.agriculture.gov.sk.ca/Default.aspx?DN=1ca)a615-f827-47ef-983e-becff77ba4f1)
- Saskatchewan Ministry of Agriculture. 2014. Guide to Crop Protection: weeds, plant diseases, and insects. Metribuzin: 218-220
- Schaefer H, P. Hechenleitner, A. Santos-Guerra, M. Menezes de Sequeira, R.T. Pennington, G. Kenicer. 2012 Systematics, biogeography, and character evolution of the legume tribe Fabeae with special focus on the middle-Atlantic island lineages. *Evolutionary Biology* 12 (250): 1-19
- Slinkard, A. E. 1981. Eston lentil. *Canadian Journal of Plant Science*, 61(3): 733-734.
- SPG (Saskatchewan Pulse Growers). 2000. Lentil production manual. Saskatchewan Pulse Growers, Saskatoon. Available at: http://www.legumematrix.com/images/563/Lentil_Manual_Saskatchewan.pdf
- SPG (Saskatchewan Pulse Growers) Annual Report. 2011. Available at: http://spg.bekinetic.ca/publications/publications/SPG_Annual_Report_2010--11/
- Si, P., B. Buirchell, and M. Sweetingham. 2009. Improved metribuzin tolerance in narrow-leafed lupin (*Lupinus angustifolius* L.) by induced mutation and field selection. *Field Crops Research*, 113: 282-286.
- Stephanova, S., P. Dolezelova, L. Plihalova, M. Prokes, P. Marsalek, M. Skoric, and Z. Svobodova. 2012. The effects of metribuzin on early life stages of common cap (*Cyprinus carpio*). *Pesticide Biochemistry and Physiology*, 103(2): 152-158.
- Tullu A., A. Diederichsen, G. Suvorova, A. Vandenberg. 2011. Genetic and genomic resources of lentil: status, use and prospects. *Plant Genetic Resources*, 9(1): 19-29.
- U.S. Environmental Protection Agency Office of Water (4607M). July 2003. Contaminant candidate list regulatory determination support document for metribuzin.
- Vandenberg A., and A.E. Slinkard. 1990. Genetics of seed coat colour and pattern in lentil. *Journal of Heredity*, 81:484-488

- Whitcomb, C.E. 2015. An introduction to ALS-inhibiting herbicides. *Toxicology and Industrial Health*, 15: 232–240.
- Wong, M.M.L., N. Gujaria-Verma, L. Ramsay, H.Y. Yuan, C. Caron, M. Diapari, K.E. Bett. 2015. Classification and characterization of species within the genus *Lens* using genotyping-by-sequencing (GBS). *Plos One*, 10(3), e0122025.
<http://doi.org/10.1371/journal.pone.0122025>

APPENDIX 1 INDOOR METRIBUZIN SCREENING PROTOCOL

1. Planting Materials and Chamber Settings

- 4 inch pots
- Soil media (this formula is made by measuring out each contents in 5 gallon buckets....60/40 mix)
 - o 2 bags of Sunshine Mix #3 (SS3)
 - o 5.5 bags of sand
 - Approximately 110 liters of sand
 - The previous bags of sand were each 18L, now there are 20L
- Chamber will be set at 14/10h day/night and at 21/15 degree Celsius day/night
- Each pot is assigned a unique pot number.
- In my experiments, there were three reps of the experiment (22 varieties, 5 rates).
 - o 22 rows
 - o 15 columns
 - o 5 rates/rep
 - o buffer on outside edges to shield off thrips
 - o 3 reps (represented by different colors)
 - o



2. Soil Mixing

- use large mixer (access key available from phytoron staff)
- place 1 bag of SS3, then three bags of sand, then 1 bag of SS3 and then remaining 3 bags of sand.
- Start mixer and let run until soil and sand is evenly mixed (I let the machine run about 5 minutes)
- Place wheel barrow under machine to let mixture out

- When the mixture is getting low, turn on the machine to help load the wheel barrow

3. Potting

- Fill pots completely with the mixture
 - Do not the mixture
 - When cart is full, take a 4-inch pot and press lightly on each soil filled pot to create an impression
 - This makes it easier to water, as the water will not spill over from pot to pot.
- Fill chamber with pots
- Add 100ml of water to each pot and allow to soak into the mixture
- Make 4 holes for seeding, about 0.5 inch deep
 - I used my index finger, go until first knuckle

4. Seed Source

- Gather seed source
- Nick each seed with a razor

5. Seeding

- Place labels in pots
- Seed each pot with four seeds, then cover with soil mixture that is in pot
 - The day before spraying, weed down to 2 plants, based on node stage and uniformity
- ***I have noticed it is better to plant seeds just before the lights go off in the chamber. My lights go off around 10p, so I started seeding around 7pm, allowing the seeds to soak up the moisture overnight. With this method of nicking and timing of seeding, I have had nearly 100% germination and great uniformity

6. Nutrient Solution

- ¼ strength Hoaglands nutrient mixture

Method for Quarter Strength Hoaglands

Solution

Make up Nutrient mix solution according to amounts required in table below - Note amounts (mls) for 5,10 or 20 litres

Check all solutions pH needs to be between 6-7

Stock Solutions	5lt mix	10 lt mix	20 lt mix
KNO ₃ potassium nitrate	6.25	12.5	25
Ca(NO ₃) ₂ calcium nitrate	6.25	12.5	25
MgSO ₄ .7H ₂ O magnesium sulphate	2.5	5	10
KH ₂ PO ₄ potassium di-hydrogen orthophosphate	1.25	2.5	5
Fe EDTA	1.25	2.5	5
Micronutrients	1.25	2.5	5

7. Water Schedule (HS= Hoaglands Solution)

Day	action	type	amount	Scoring
Day 0	sowing			
	water	water	100ml	pre sowing
Day 3	water	water	100 ml	
Day 8	water	Quarter HS	100ml	
Day 10	water	quarter HS	100ml	
Day 12	Water	quarter HS	50ml	0 day spray height, node stage
Day 13	Water	quarter HS	50ml	
Day 15	Water	quarter HS	100 ml	
Day 18	notes			7 day height & % necrosis
Day 21	water	quarter HS	100 ml	
Day 24	water	quarter HS	100 ml	
Day 25	notes			14 day height & % necrosis
Day 27	water	quarter HS	100 ml	
Day 31	water	quarter HS	100 ml	
Day 33	notes			21 day height, % necrosis & biomass cut

- Recent studies have shown that plants appear to be healthiest when watering at 50ml, every 3 days when plants emerge

Day	action	type	amount	Scoring
Day 0	sowing			
	water	water	100ml	pre sowing
Day 3	water	water	100 ml	
Day 8	water	quarter hoags	50ml	
Day 11	water	quarter hoags	50ml	Initial spray height, node stage
Day 12	Water	quarter hoags	50ml	
Day 15	Water	quarter hoags	50ml	
Day 18	Water	quarter hoags	50 ml	
Day 18	notes			7 day height & % necrosis
Day 21	water	quarter hoags	50 ml	
Day 24	water	quarter hoags	50 ml	
Day 25	notes			14 day height & % necrosis
Day 27	water	quarter hoags	50 ml	
Day 31	water	quarter hoags	50 ml	
Day 33	notes			21 day height, % necrosis & biomass cut

8. Notes for Success

- Reduce number of plants down to 2 on day before spraying
- Color coding tags for spray rates helps with spray order
- Spray down the chamber after every watering to remove soil from floor
- Tape down bottom of door on outside, will help keep thrips out
- Only enter chamber when needed (watering, notes)
- Do not let anyone else enter the chamber during the experiment

APPENDIX 2

Root Growth Observations

Profile® Greens Grade™ is a profile porous ceramic inorganic soil mixture. The soil resists compaction and allows for healthy root development, and can be an alternative for peat (Profile®, 2015). This ceramic has 74% pore space, allowing for increased drainage, yet holding moisture for plant roots as needed (Profile®, 2015). This soil media has been used for growth room projects at Colorado State University, Fort Collins, CO.

Current indoor experiments for lentil are typically grown in a 60/40 Sunshine 3 and sand mixture. Concerns have arisen that high organic matter of Sunshine 3 mix may interfere with metribuzin uptake, and that there is no traceable quality control of the organic material. This can contribute to variability of experimental results for some types of experiments.

Profile® Greens Grade™ was used as a growth medium for lentil in a growth chamber experiment in the phytotron. The porous mixture was compared with the 60/40 Sunshine 3 and sand mixture using the working metribuzin screening protocol (Appendix 1). Each soil medium was placed in 4 inch pots which were sown with sufficient lentil seed to allow thinning to 4 lentil plants per pot. The trial was included with a running dose response, and received the same rates of metribuzin as reported in Experiment 3.

The lentil plants emerged well in both soil media. However, the seeds sown in the Profile® Greens Grade™ would pop up above the surface during germination and emergence. The seed was planted at 2.5cm deep, as prescribed in the working protocol (Appendix 1), but the seed cotyledons were pushed above the surface of the primary root development. The seed displacement caused seedlings to lodge as they continued to grow.

A second observation related to the use of the ceramic growth medium was noticed when the plants were thinned down to 2 plants per pot prior to spraying metribuzin. When plants were pulled the Sunshine mix/sand medium there was a single root shoot with minimal branching. Roots pulled from the porous mixture had a long tap root and several lateral branches, and appeared to be much healthier based on visual observations (Figure A.2.1).

Based on these observations, Profile® Greens Grade™ should be further explored as a potential soil medium for indoor growth room projects. The plants appeared to be more vigorous and healthier. This may also help minimize problems with soil borne thrips and be beneficial for herbicide screenings because of the reduced amount of organic matter.



Figure A.2. 1 These four plants of CDC Greenstar were planted on the same day. The two plants on the left were grown in a 60/40 Sunshine Mix # 3 and sand mixture. The two plants on the right were grown in Profile® Greens Grade™.